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WORKING REPORT SERIES (BAFFIN ISLAN
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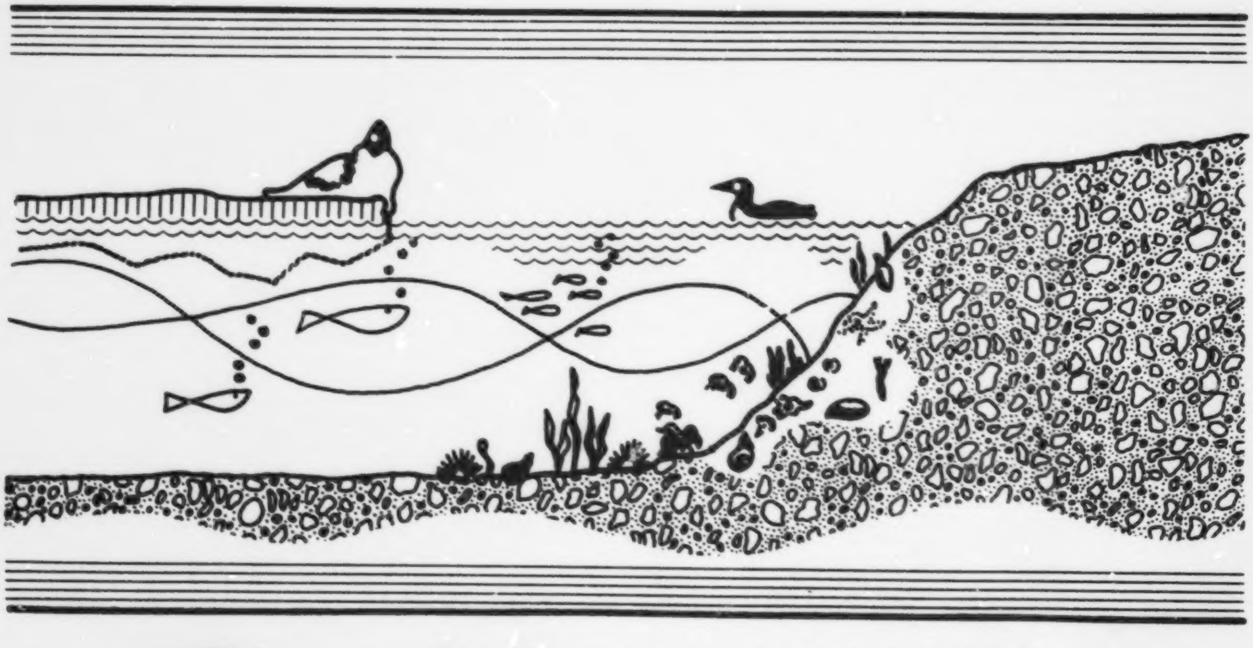
SPECIAL STUDIES



Baffin Island Oil Spill Project

WORKING REPORT SERIES

1981 STUDY RESULTS

81- 10

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FOREWORD

SPECIAL STUDIES 1981

This publication presents the results of studies which were carried out in 1981 at the BIOS Project site of Cape Hatt, Baffin Island, but which were ancillary to the original design and core programs of the Project. Although deemed to be worthwhile contributions to the Project and oil spill studies generally, the BIOS Management Committee did not directly fund these studies due to financial limitations.

Two reports are contained herein. The first presents the results of tissue analysis on two species of benthic fauna. This data is additional and complementary to the results in the BIOS Working Report 81-2: ANALYTICAL BIOGEOCHEMISTRY. The second report presents the results from the first year of a two-year study on under-ice biota.

Correct citations for this publication are as follows:

Engelhardt, F.R. and Norstrom, R.J., 1982, Petroleum hydrocarbons in two benthic invertebrates, the urchin Strongylocentrotus droebachiensis and the polychaete Pectinaria granulosa. IN: Special Studies - 1981 Study Results. (BIOS) Baffin Island Oil Spill Working Report 81-10.

Cross, W.E., 1982, In Situ studies of effects of oil and dispersed oil on primary productivity of ice algae and on under-ice amphipod communities. IN: Special Studies - 1981 Study Results. (BIOS) Baffin Island Oil Spill Working Report 81-10.

BAFFIN ISLAND OIL SPILL WORKING REPORT
1981 STUDY RESULTS
SPECIAL STUDIES

1. Petroleum Hydrocarbons in Two Benthic Invertebrates, the Urchin *Strongylocentrotus Droebachiensis* and the Polychaete *Pectinaria Granulosa*

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2. In Situ Studies of Effects of Oil and Dispersed Oil on Primary Productivity of Ice Algae and on Under-Ice Amphipod Communities

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BAFFIN ISLAND OIL SPILL PROJECT

Report on the 1981 Oil Spill Experiment

- Chemistry Component (Part B) -

PETROLEUM HYDROCARBONS IN TWO BENTHIC INVERTEBRATES, THE URCHIN *STRONGYLOCENTROTUS DROEBACHIENSIS* AND THE POLYCHAETE *PECTINARIA GRANULOSA*

Final Report

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September 1982

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SECTION ONE - INTRODUCTION

1.1 Spill Scenario

The design of the experimental oil spill protocol in the Baffin Island Oil Spill (BIOS) Project called for the discharge of chemically dispersed oil and untreated crude oil into various bays in Ragged Channel, Cape Hatt, N.W.T. (Green et al. 1982). A spill of $15m^3$ (75 drums) of slightly aged oil (7% air-stripped Lagomedio crude) was carried out in Bay 11 at 1540 on 19 August 1981. An estimated 37 drums of oil were collected from the surface one day after the spill. Bay 9 was treated at 1300 on 27 August 1981 with a sub-surface diffusion of $15m^3$ of aged Lagomedio crude. This oil was chemically dispersed using 10% Corexit 9527.

The concentration of oil in the water column was measured by Seakem Oceanography Ltd. using real-time fluorometry. Oil was found to be distributed in the two spill bays, as well as two other test bays, Bays 10 and 7. On the basis of 36 hours of monitoring, water column concentrations were found to be several hundred ppm-hrs. in Bay 9, tens of ppm-hrs in Bay 10, less than 1 ppm-hr in Bay 7, and several ppm-hrs in Bay 11. The details of the spill scenario and real-time water column data can be found in Green et al. (1982). Further data on water column samples, as well as of sediment and shoreline substrates can be found in a report by Energy Resources Company Inc. - ERCO (Boehm et al. 1982). Some of this physical information will be discussed later in reference to the findings of this study.

1.2 Chemistry Study Design

Part of the 1981 BIOS Project study was a detailed identification of petroleum hydrocarbon concentration and composition in benthic biota. Seven species of in- and epifaunal invertebrates were collected at selected depths along defined transects in each of the four test bays. Five of the species were bivalves, Mya truncata, Serripes groenlandica, Macoma calcarea, *Astarte borealis* and *Nuculana minuta*. Tissue hydrocarbon loads in these species were analyzed under contract by ERCO (see Boehm et al. 1982). Two other species, the sea urchin *Strongylocentrotus droebachiensis* and the tube-dwelling polychaete *Pectinaria granulosa*, were analyzed by the National Wildlife Research Centre, Canadian Wildlife Service. This work was carried out in conjunction with Northern Environmental Protection, Indian and Northern Affairs, through the collegial association of the authors of this report. The assistance of M. Wong is gratefully acknowledged.

Analytical methodologies and instrumentation were standardized to a large degree, permitting ready comparison of data originating from both the CWS/DIAND and ERCO laboratories. An intercalibration exercise between the laboratories served as a further check on data consistency.

Hydrocarbon analysis was carried out also in 1980 on several species of plants and animals from the spill test area, including *M. truncata* and *S. droebachiensis*. The results of this work have been reported separately (Boehm 1981). In summary, both species showed evidence of hydrocarbons in body tissues, but this was predominantly of biogenic origin. Very low (ppb) and uniform levels of hydrocarbons of petroleum origin were also recorded, forming a trace level baseline.

SECTION TWO - MATERIALS AND METHODS

2.1 Sampling Protocol

A detailed description of the sampling protocol and methods for benthic biota is presented in a report by LGL Limited (Cross and Thomson 1982). For purposes of this study, both urchins and polychaetes were collected in 1981 from each of the four test bays, on three occasions: before the oil discharges, one to four days after the discharge, and 14 to 20 days post-spill.

Both S. droebachiensis and P. granulosa were sampled from each of five sampling stations at the 7 m depth stratum of the tissue transect. Ten or more individuals were scheduled to be taken from each station. Additionally, a group of urchins was kept in screen enclosures (traps) at the 5 m depth, and sampled similarly.

After collection, all samples were wrapped in aluminum foil, frozen, and stored until dissection and analysis.

2.2 Analytical Methods

2.2.1 Tissue Processing

All individuals were dissected while still frozen in order to minimize loss of cellular fluids liberated during the freezing process, as well as volatile hydrocarbon losses. All dissecting implements and glassware were rinsed with pesticide grade acetone prior to contact with any sample. Only soft tissues were processed for analysis. In the case of S. droebachiensis, the test was scraped internally to free soft tissue adhesions, and discarded along with the mouthparts. Care was taken to avoid contamination of the sample by material on the external surface of the urchin test. The P. granulosa were removed from their tubes and the entire soft animal was processed.

Pooled samples of P. granulosa from 7 m stations were found to be too small individually to allow analysis (ca. 1g or less). All five samples from a depth stratum for each bay and one time point were pooled. Subsequent procedures were the same as for S. droebachiensis.

Prior to frozen storage of the homogenized pooled samples, an aliquot of each homogenate was dried overnight at 200°C for determination of water content and dry weight calculations.

2.2.2 Extraction and Cleanup

The method of alcoholic KOH saponification followed by extraction was adopted in favour of steam distillation because apparatus of a suitable scale for analysis of small samples using the latter procedure was not available. The main potential disadvantage of the saponification procedure is that removal of the large volumes of solvent employed in extraction and cleanup entails losses of the more volatile components. In order to minimize this problem, a procedure was developed which required only one evaporation step prior to screening by GC^2FID (gas chromatography with flame ionization detectors), and one more subsequent to addition of the aromatic internal standard (o-terphenyl). A flow chart of the method is given in Fig. 2.1 and the procedure is given in detail below.

A single alumina column cleanup step was found to be sufficient for analysis of n-alkanes, pristane and phytane by FID in spite of the relatively large quantities of biogenic compounds present. The latter, partly identified as squalenes (hexacosaene isomers) and long-chain fatty acid alkyl esters by GC/mass spectrometry, occurred in the C20-28 region of the chromatogram, but were resolved from the n-alkanes. Similarly, there were no significant interferences from alkanes, olefins or biogenic material in the analysis of aromatics by GC^2/SIM (gas chromatography with single ion monitoring) so that separation into F1 and F2 fractions using silica gel chromatography was not required.

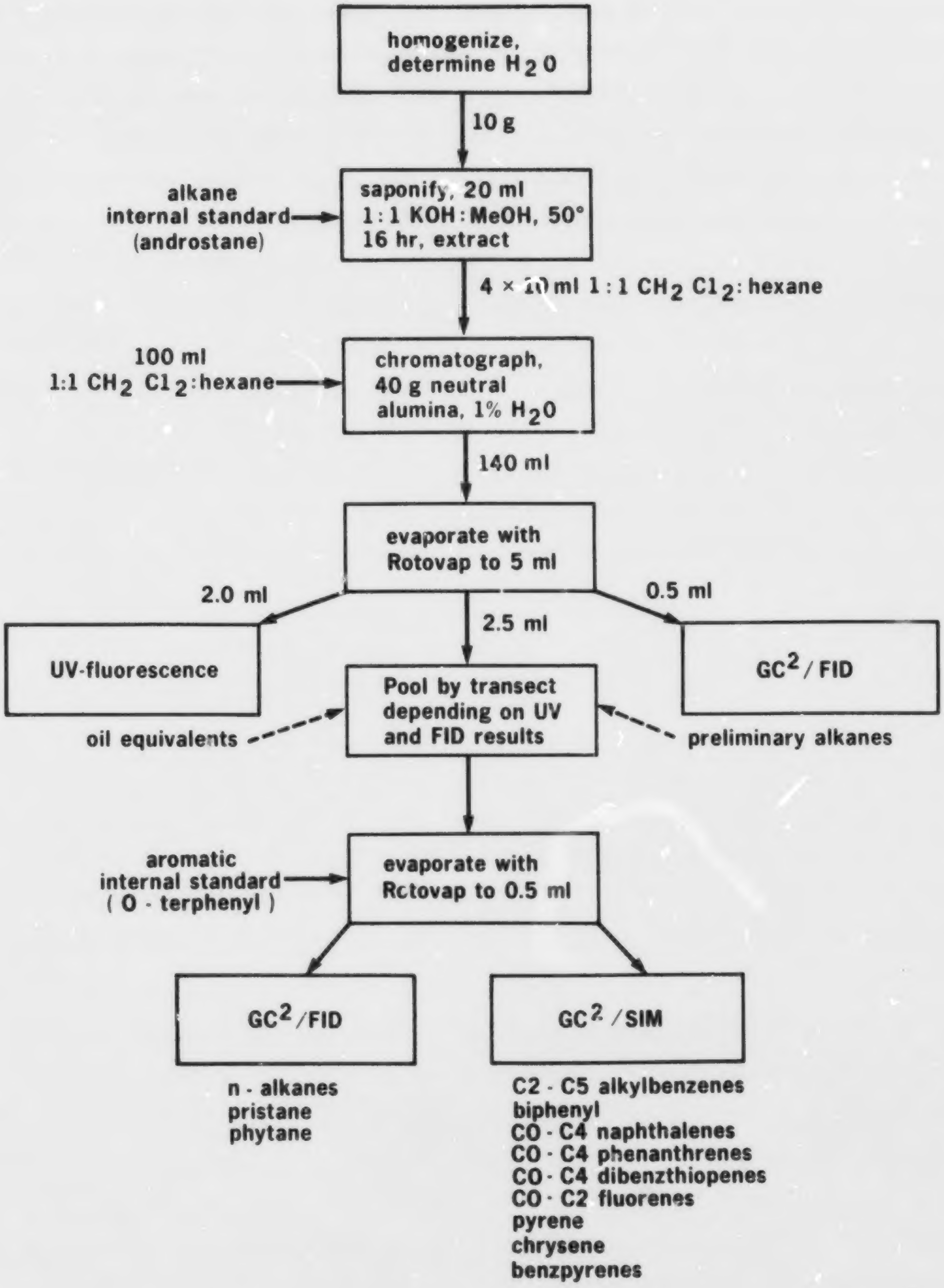

homogenize, determine H2O
10 g
alkane internal standard (androstane)
saponify, 20 ml 1:1 KOH:MeOH, 50° 16 hr, extract
4 × 10 ml 1:1 CH2Cl2:hexane
100 ml 1:1 CH2Cl2:hexane
chromatograph, 40 g neutral alumina, 1% H2O
140 ml
evaporate with Rotovap to 5 ml
2.0 ml
2.5 ml
0.5 ml
UV-fluorescence
GC2/FID
Pool by transect depending on UV and FID results
oil equivalents
preliminary alkanes
aromatic internal standard (O - terphenyl)
evaporate with Rotovap to 0.5 ml
GC2/FID
GC2/SIM
n - alkanes
pristane
phytane
C2 - C5 alkylbenzenes
biphenyl
CO - C4 naphthalenes
CO - C4 phenanthrenes
CO - C4 dibenzthiopenes
CO - C2 fluorenes
pyrene
chrysene
benzpyrenes

Figure 2.1 Extraction and cleanup schedule preparatory to GC^2/FID and GC^2/SIM analyses

Samples from each station were thawed and re-homogenized by shaking the vial vigorously. A 10 g sample (or sample plus water to make 10 g) was pipetted into a 50 ml centrifuge tube with teflon-lined screw cap, followed by the internal standard (0.2 ml, 10^{-4} g/ml androstane in hexane), and 20 ml of 1:1 methanol: 10N KOH. The sample was capped tightly, shaken, and digested in a 50°C waterbath shaker for 16 hr. After cooling in the refrigerator for ½ hr., the tube was opened and 10 ml of 1:1 dichloromethane:hexane were added immediately. The sample was vortex mixed, centrifuged and the top layer transferred to a flask. This procedure was repeated a minimum of 4 times, or until the organic layer was colourless. The combined extracts were placed directly on the alumina clean-up column (40 g Fisher A950 neutral alumina, wet packed with 1:1 dichloromethane:hexane. The eluate was evaporated to ca. 2 ml with a rotary evaporator, transferred to a 15 ml centrifuge tube and made up to 5 ml with hexane. The sample was partitioned as follows: 0.5 ml for GC^2/FID analysis, 2.0 ml for fluorescence analysis, and 2.5 ml for GC^2/SIM analysis after pooling. All solvents and reagents used in processing were of ultra-pure grade.

2.2.3 UV/Fluorescence Analysis

The UV/F analysis was carried out on clean extracted samples processed as described above. Fluorescence determination were carried out using a Turner Model 430 fluorometer at fixed wavelengths (excitation 300 nm, emission 350 nm, slit widths 15 nm).

The choice of wavelengths was predicated on needs for maximum absolute sensitivity, for realistic representation of total tissue hydrocarbon load, and for inter-convertibility of data originating from ERCO and the DIAND/CWS association. Spectral scans of aged (7% air-stripped) Lagomedio crude oil standards in hexane showed excitation peaks at 300 and 355 nm, although fluorescence was generally high between these two peaks. An emission maximum occurred at 405 nm, peaking from a gradual rise.

A better link with actual tissue hydrocarbon load was sought by verification of the emission fluorescence spectra of selected aromatic hydrocarbons, excited at 300 nm. The following relevant excitation peaks were found: benzene, 345 nm; naphthalene, 337 nm; phenanthrene, 364 nm; benzanthracene, 411 nm; benzopyrene, 409 and 429 nm. These aromatic hydrocarbons were pure compounds dissolved as 5 ng/ml in dichloromethane and hexane, 1:1. A survey of the GC^2/SIM data showed that tissue hydrocarbon compositions emphasized the two- and three-ringed aromatic structures which individually tended to have emission maxima closer to 350 nm than the 405 nm peak of the crude oil hydrocarbon mixture. This suggested an optimum choice for emission wavelengths in the mid-300's. Further, the decision had been made in the ERCO laboratory to use 325 and 350 nm as its quantitation settings.

Using a 300/350 nm combination, calibration was carried out using hexane as a blank and Legomedio crude oil in hexane as a standard. This was verified against the use of dichloromethane - hexane, 1:1 as a diluant which was not found to differ in its blank values. A calibration range of 0-15 ng/ml was used, a range in which the fluorescence readings were nearly linear and not limited by self-absorption. Calibration curves were determined, against which unknown and check samples could be read for concentration. Many of the tissue extracts required dilution with hexane to fit them into the calibration range. All solvents were of ultra pure grade. Final concentrations of tissue total hydrocarbons were expressed as ng/g dry weight on the basis of the crude oil standard.

2.2.4 GC^2/FID Analysis

This analysis was performed with a Hewlett-Packard 5840 GC equipped with an autoinjector and 30 m fused silica column (DB-5, 0.25 mm i.d., 0.1 micron film thickness, J&W Scientific). Injections were performed automatically in the splitless mode, programming from 60°C to 300°C at 5°/min. The injector was at 250°, and the detector at 300°. The carrier gas was helium at 20 psi head pressure. The recovery of androstane was calculated by comparison to the area of external standards made directly from the spiking solution, and injected in the same series of runs.

The majority of samples did not contain significant quantities of n-alkanes. For those samples which contained alkanes, the procedure adopted was to calculate oil equivalents based on the sum of peak heights of the C16-26 n-alkanes. The standard used for this calculation was prepared from an aliquot of oil put through the entire analytical procedure (except saponification). In addition, the distribution of n-alkanes relative to that in the oil was plotted (vide infra) for these samples. Pristane, which was a major biogenic hydrocarbon in S. droebachiensis, and phytane were determined using external standards. All results were corrected to androstane recovery.

2.2.5 GC^2/SIM Analysis

This analysis was performed with a Hewlett-Packard 5985B GC/MS equipped with a 30 m fused silica column (SE-54, 0.25 mm i.d., J&W Scientific). Injections were performed manually in the splitless mode using a temperature program and GC conditions identical to those for GC^2/FID analysis. The column was inserted through the interface directly into the ion source. The source was at 200°C. and electron energy was 70 ev.

An early version of HP's 100 ion SIM software was used for analysis. The sensitivity was sufficient for analysis of the levels of aromatic hydrocarbons found in the samples. Table 21 lists the compounds determined, the molecular ion (used in all cases), and the ion group in which the ion was employed. The latter is a procedure whereby the chromatogram was divided into 5 time segments, in each of which only 7 ions were scanned with a dwell time of 10 msec per ion. This procedure enhanced sensitivity, and the ability to detect and accurately quantitate narrow peaks.

Table 2.1 Summary of single ion monitoring (SIM) condition employed for analysis of aromatic hydrocarbons by GC^2 mass spectrometry

Compound or compound group	Abbreviation	Monitored ion	Present in ion group number
C2 alkyl benzenes	C2AB	106	1
C3 alkyl benzenes	C3AB	120	1
C4 alkyl benzenes	C4AB	134	1
C5 alkyl benzenes	C5AB	148	1
biphenyl	BP	154	2
naphthalene	N	128	1
C1 naphthalenes	CIN	142	1
C2 naphthalenes	C2N	156	2
C3 naphthalenes	C3N	170	2
C4 naphthalenes	C4N	184	2
fluorene	F	166	2
C1 fluorenes	CIF	180	3
C2 fluorenes	C2F	194	3
phenanthrene	Ph	178	3
C1 phenanthrenes	C1Ph	192	3
C2 phenanthrenes	C2Ph	206	4
C3 phenanthrenes	C2Ph	220	4,5
C4 phenanthrenes	C4Ph	234	5
dibenzthiophene	BP	184	3
C1 dibenzthiophenes	C1BP	198	3
C2 dibenzthiophenes	C2BP	212	4
C3 dibenzthiophenes	C3BP	226	4
C4 dibenzthiophenes	C4BP	240	4,5
pyrene	Py	202	4,5
chrysene, benzanthracene	Chr	228	4,5
benzopyrenes	BPy	252	5
o-terphenyl (intern.std.)	IS	230	2,3

Samples with unusually high contamination as determined by UV fluorescence were analyzed individually. Otherwise, samples from a given Bay and time point were pooled by transect, the o-terphenyl internal standard (5 ug) added, and the volume (generally 12.5 ml) reduced to 0.5 ml in a small flask using a rotary evaporator. Each sample was re-analysed by GC^2/FID at this point to obtain the final pristane, phytane and n-alkane results. The aromatic hydrocarbons were quantitated as o-terphenyl equivalents by measurement of areas for each ion in the correct retention time range for that compound class as determined from an authentic standard of the oil.

2.3 Methodology Validation and Quality Assurance

2.3.1 UV/F

The reproducibility of the fluorescence assay was tested by a series of duplicate analyses of tissue and oil extracts (n = 7). The error was found to average 3.2 ± 0.9%. Recovery as expressed by the fluorescence data was assessed using four oil standards of 100 to 200 ng/ml. Recovery was found to be 70 ± 4%, and the data presented in Section 3 (Results) are not corrected for extraction losses.

The suitability of the 300/350 nm wavelength settings was confirmed by spectral scans of extracted urchin samples. Excitation fluorescence peaks were found at 300 and 360 nm, and emission peaks broadly at 360 and 400 nm.

2.3.2 GC^2/FID

The procedure for alkane analysis was tested by spiking oil to 10 ml of water at the 100 and 200 ug/ml level; to 10 g of clean, pre-spill *S. droebachiensis* homogenate at the 100 and 200 ug/ml levels in duplicate, and to 10 g *P. granulosa* homogenate at the 200 ug/ml level in duplicate. These samples were carried through the entire procedure and the recovery of n-alkanes was calculated by FID determination against an external standard of the same oil. The results are shown in Figure 2.2. The recovery above C16 was acceptable for quantitative analysis, i.e. greater than 80%, in all cases except the 100 ug/ml oil-in-water test sample. The loss of volatiles in this sample could have been due to the absence of biogenic "carrier" materials such as lipids. Biogenic hydrocarbons constituted significant interference at C23, C25 and C27 in the case of

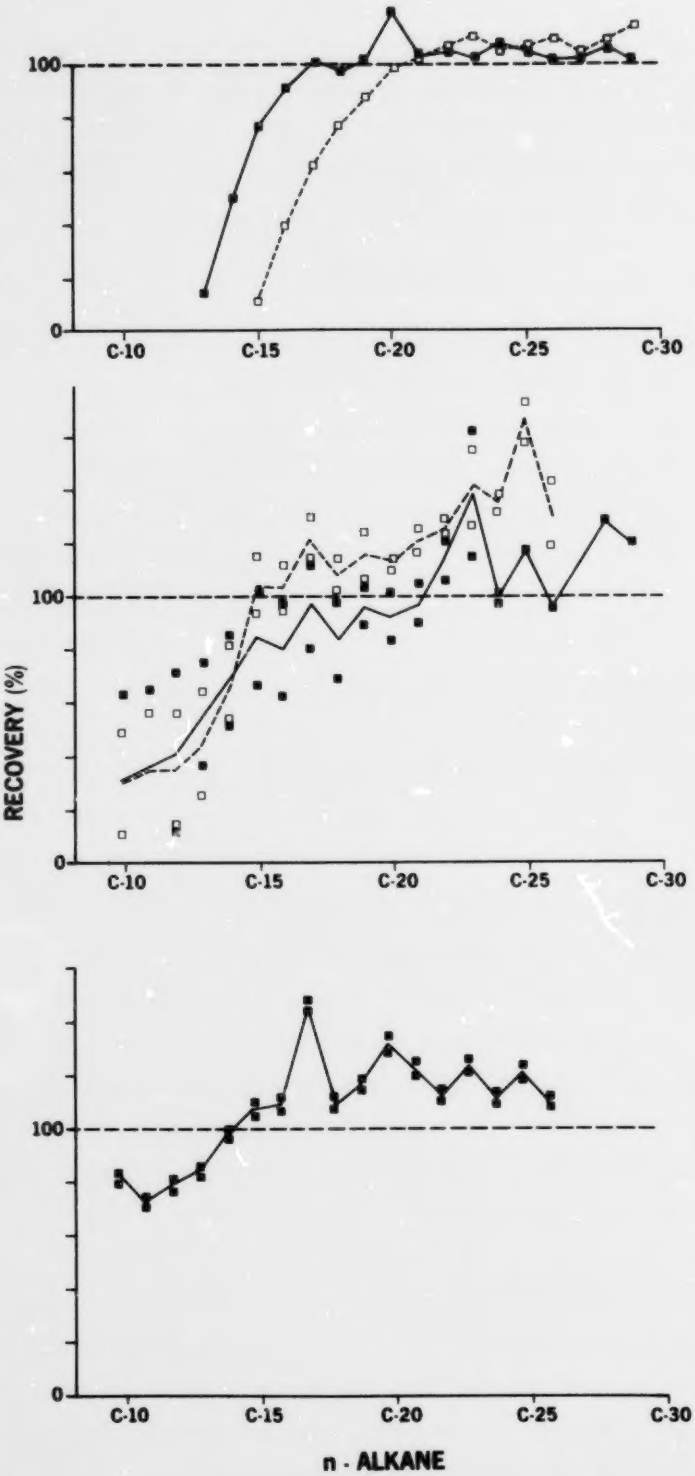


Figure 2.2 Recovery of n-alkanes by GC^2/FID analysis

the urchins, and at C17, C20, C23 and C25 in the case of the annelids. Only seven samples had significant levels of alkanes in them, and of these, only two were as low as the 100-200 ug/g range. Interference from biogenic hydrocarbons was therefore not a significant source of error. Androstane recoveries were found to be 94 ± 8% (n = 6).

2.3.3 GC^2/SIM

In order to determine recoveries of the aromatic compounds in the PAH fraction, a sample of oil was separated on silica gel into aliphatic and aromatic fractions. Pre-spill samples of urchins and polychaetes and water were spiked at the 50 or 100 ug/g oil equivalent level with the PAH fraction and carried through the whole procedure. Recovery was calculated by GC^2/SIM determination against an external standard of the same PAH fraction of the oil. The o-terphenyl internal standard was added at the saponification step for this study only. The results are presented in Table 2.2. The o-terphenyl internal standard recovery was 101 ± 4% (n = 7). It is clear from the results that there is more of a problem in the determination of volatile compounds (e.g., N and C/N) and the relatively minor constituents (e.g. C3Ph, C2F) than there is for the major constituents, which was expected. If the major constituents are identified as C4AB, C1-C4N, DBT, C1-C3DBT, and C1-C2Ph, the overall recovery was 88%.

Because of potential errors introduced by the spiking procedure itself, the precision of the method was tested by analysis in quadruplicate of an urchin sample which had been screened and found to contain significant PAH contamination. The results of this analysis are presented in Table 2.3. The coefficient of variation for the major constituents of low volatility was less than 10%, which is a satisfactory precision for determination of compounds at levels less than 1 ppm.

2.4 Laboratory Intercalibration

In order to validate the comparability of data generated by CWS/DIAND and ERCO laboratory techniques it was decided to have each laboratory team analyze a number of samples of, to them, unknown concentration. Results would be compared after completion of the exercise.

Table 2.2 Recovery of the PAH fraction, spiked at 50 and 100 ug/g in crude oil equivalents to pre-spill samples and blanks

		PERCENT RECOVERY[1]																		
SAMPLE TYPE	Spike ug Oil/ g wet wt.	IS	C4 AB	BP	N	C1 N	C2 N	C3 N	C4 N	DBT	C1 DBT	C2 DBT	C3 DBT	Ph	C1 Ph	C2 Ph	C3 Ph	F	C1 F	C2 F
S. droeb.	100	106	-	75	15	11	49	77	91	97	106	119	144	123	129	120	122	98	125	209
" "	100	99	56	106	86	90	95	94	-	84	91	94	99	107	110	98	91	86	93	144
" "	50	98	104	141	129	89	92	97	51	50	109	91	26	106	102	81	-	130	-	111
" "	50	105	97	124	89	95	89	86	61	54	28	-	-	62	63	79	-	-	-	-
P. gran.	100	96	116	110	206	83	89	83	112	102	106	111	135	114	142	71	136	-	-	-
Blank	100	104	70	80	92	72	71	72	102	87	87	89	119	106	92	110	80	103	97	148
Blank	50	96	42	85	75	67	71	72	76	84	81	87	-	111	94	102	136	-	-	-

[1] IS = internal standard (o-terphenyl), AB = Alkyl benzenes, N = naphthalene, DBT = Dibenzthiophene

Ph = phenanthrene, F = fluorene

Table 2.3 Reproducibility of the fluorescence and GC^2/SIM anlysis procedures

STR, Bay 10 1st post spill Duplicate No.	Oil Equivalents, (UV fluoresc., ug/g dry weight	ng/g dry weight[1]																								
		C3 AB	C4	C5	BP	N	C1 N	C2 N	C3 N	C4 N	DBT	C1 DBT	C2 DBT	C3 DBT	C4 DBT	Ph	C1 Ph	C2 Ph	C3 Ph	C4 Ph	F	C1 F	C2 F	Chr	Total Aromatic	Total PAH
P#1	86.6	400	463	233	45	195	557	883	1288	757	125	270	216	137	NA	355	392	280	148	20	51	95	119	40	7069	5973
P#2	92.7	263	364	230	60	153	522	919	1325	641	139	266	247	181	NA	385	41[illegible]	310	150	29	63	111	85	NA	6860	6003
P#3	95.6	607	766	347	68	215	698	1007	1503	675	134	245	210	162	34	341	380	263	156	20	63	77	153	8	8132	6412
P#4	94.2	312	553	263	53	122	511	863	1192	618	134	238	235	162	43	324	392	261	148	NA	54	76	83	NA	6637	5509
Mean	92.3	396	537	268	56	171	572	918	1327	673	133	255	227	161	39	351	395	278	151	23	58	90	108	-	7175	5974
SD (±)	4.0	152	171	55	10	42	86	64	130	61	6	16	17	18	7	26	16	23	4	5	6	17	32	-	662	369
CV (%)	4	38	32	20	17	24	15	7	10	9	4	6	7	11	11	7	4	8	2	23	11	19	30	-	9	6

[1] AB = alkyl benzene
BP = biphenyl
N = naphthalene
DBT = dibenzthiophene
Ph = phenanthrene
F = fluorene
Chr = chrysene

The set of intercalibration samples were prepared by a chemist not associated with these analyses by spiking a clam (M. truncata) extract and an urchin (S. droebachiensis) extract with oil. In addition, a solution of Lagomedio crude oil in hexane at an unknown concentration was prepared. The biological samples were carried through the normal procedures in duplicate and oil equivalents calculated by comparison to an external standard of the same oil. The unknown oil sample was analysed directly.

SECTION THREE - RESULTS

3.1 Crude Oil Hydrocarbon Characteristics

Standards of the aged Lagomedio crude oil, as used in the oil spill study, were analyzed for hydrocarbon characteristics by UV/F, GC^2/FID and GC^2/SIM methods. The UV/F survey was carried out to optimize wavelength choice for tissue total hydrocarbon concentration and has been described in Section 2.2.

The chromatogram produced by GC^2/FID analysis of Lagomedio crude shows a very typical and regular composition of n-alkanes from C10 to C32 (Figure 3.1). Although the method as used without fractionation is less suitable for characterizing other hydrocarbons including aromatics, these are indicated as smaller peaks between the regular n-alkanes. A relatively large proportion of low to middle boiling fractions is evident from the chromatogram.

A fine definition of the quantitatively smaller aromatic component of Lagomedio crude was achieved by GC^2/SIM analysis. Figure 3.2 shows a combined total ion chromatogram for the crude oil, a figure generated from the summed data obtained by SIM analysis. This combined chromatogram will serve as a handy reference for the other combined chromatograms depicting tissue hydrocarbons as determined by GC^2/SIM. Single ion characteristics for the crude oil are detailed in Figure 3.2 to 3.4.

3.2 Tissue Hydrocarbons

3.2.1 Strongylocentrotus droebachiensis

3.2.1.1 UV/F

Figure 3.5 shows that the total oil burden as assessed by the UV/F method in urchins from all four bays increased within one to four days of the spill event from very low background or pre-oil levels. These increased by two weeks in Bay 9 and Bay 11 urchins to about 250 and 500 ug/g (oil equivalents per gram dry weight of soft tissue). Bay 7 and Bay 10 samples showed little increase from the first post-spill sample.

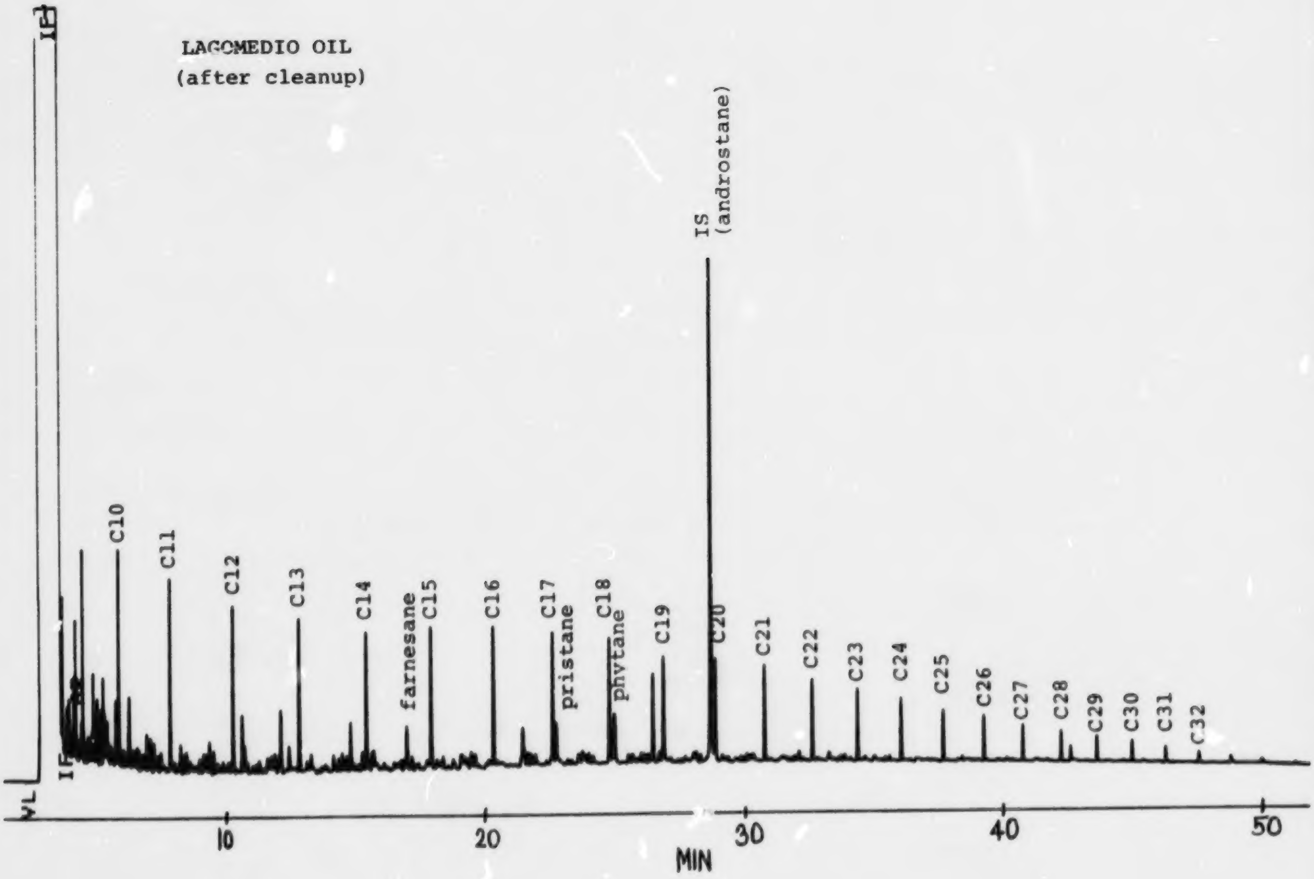


Figure 3.1 Hydrocarbon composition of Lagomedio crude oil as assessed by GC^2/FID

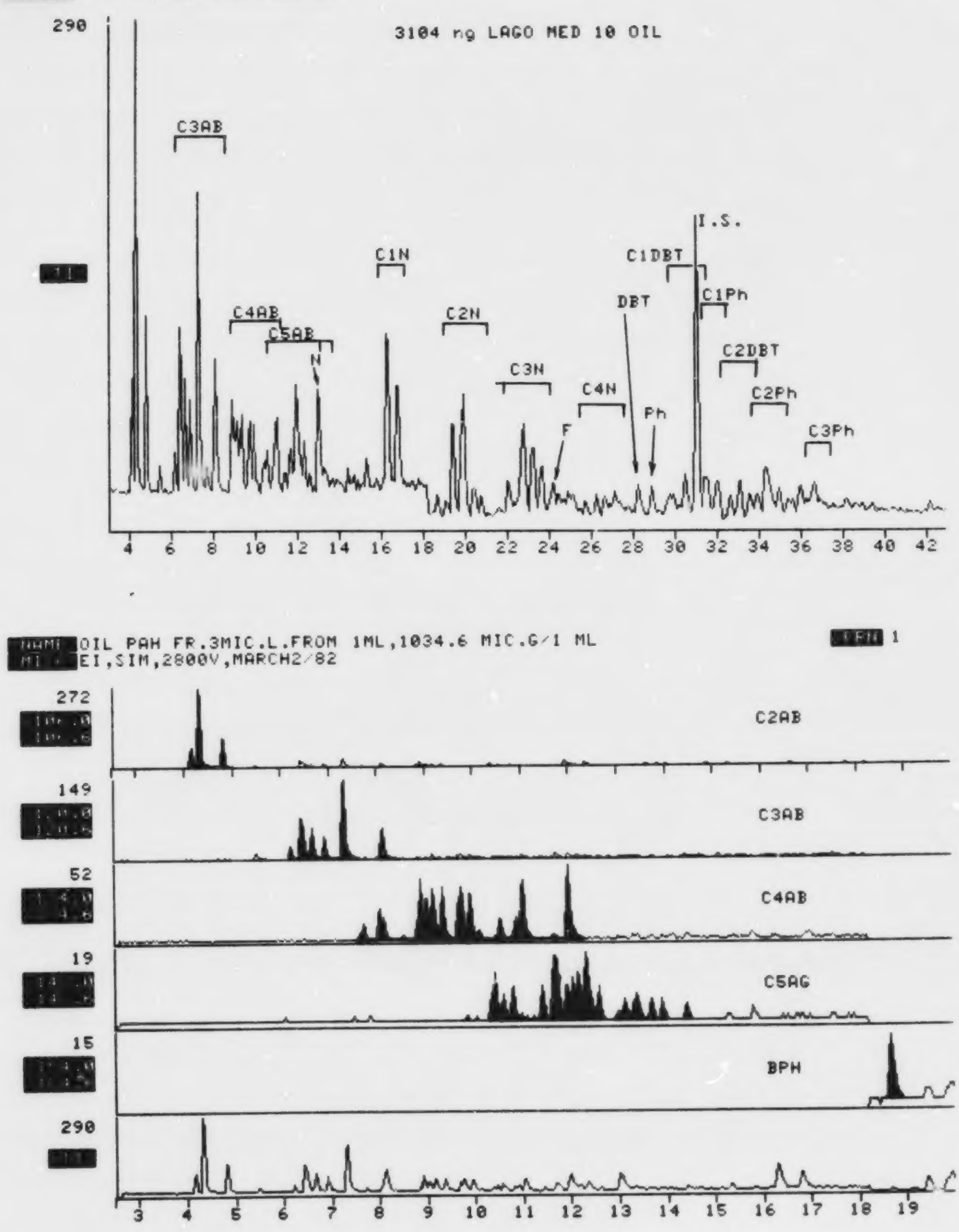


Figure 3.2 GC^2/SIM chromatograms of Lagomedio crude oil, showing a total ion chromatogram (top) and benzene to biphenyl single ion chromatograms (bottom)

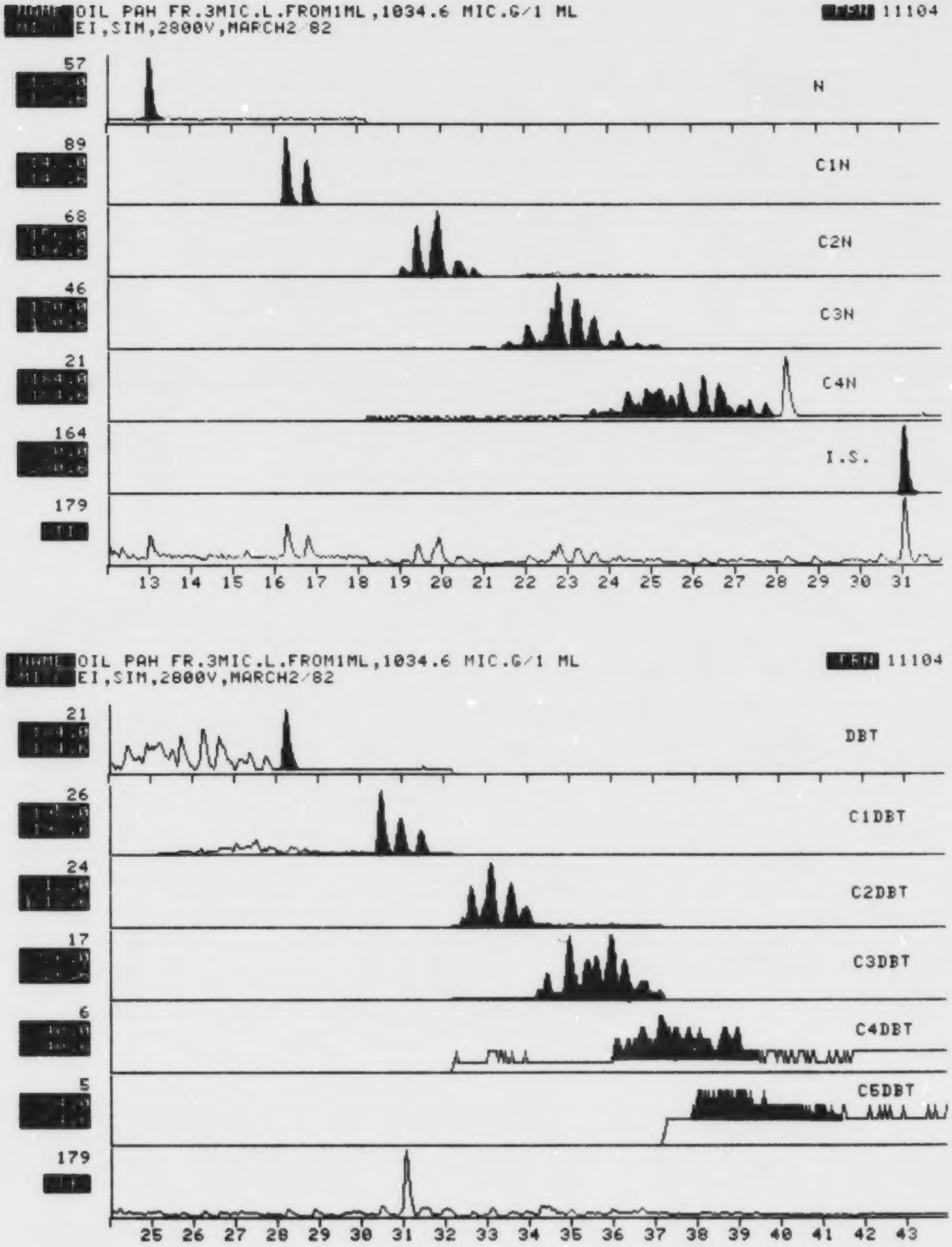


Figure 3.3 GC^2/SIM chromatograms for Lagomedio crude oil, showing naphthalenes and dibenzthiophenes as single ions

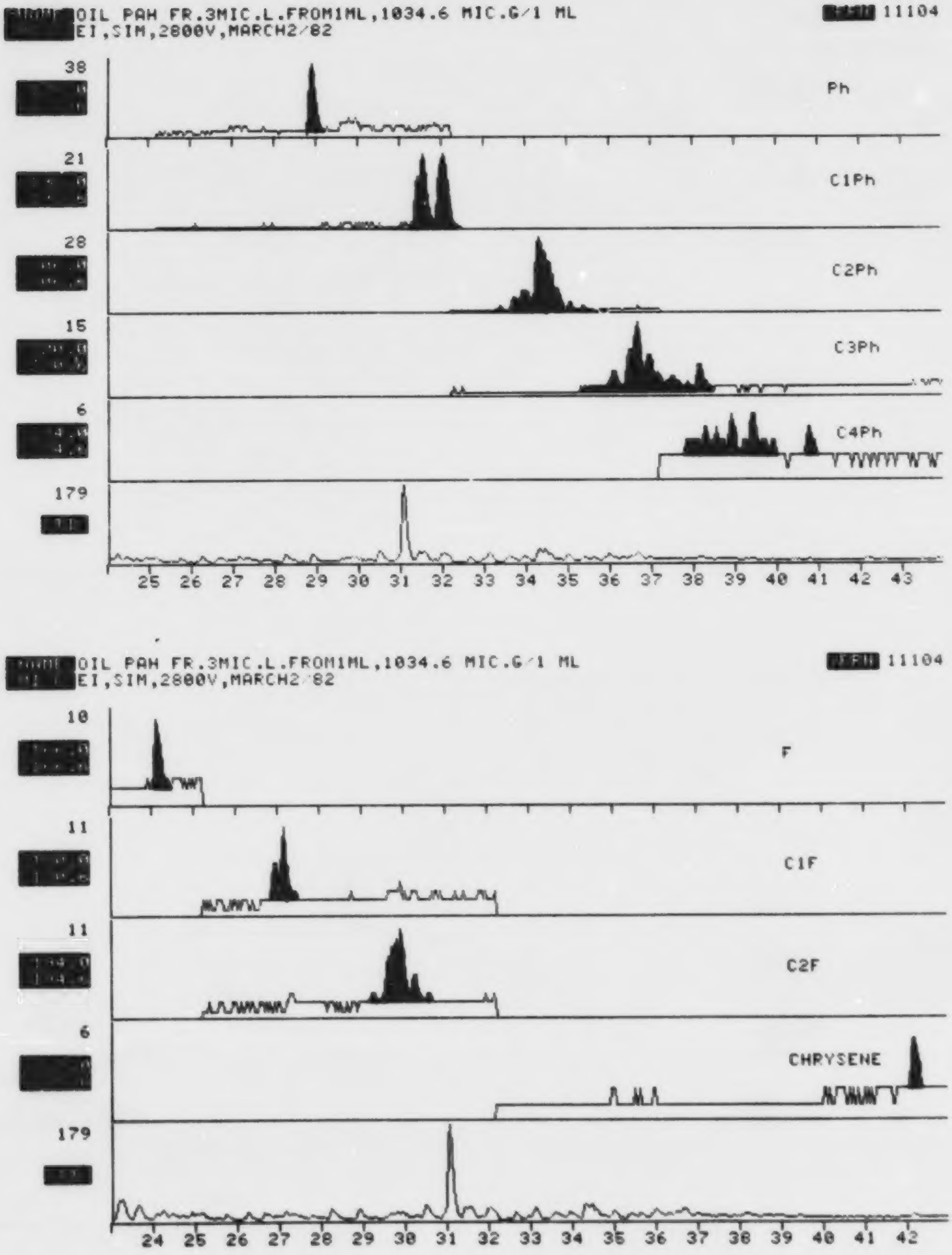


Figure 3.4 GC^2/SIM chromatograms for Lagomedio crude oil, showing phenanthrenes, fluorenes, and chrysene as single ions

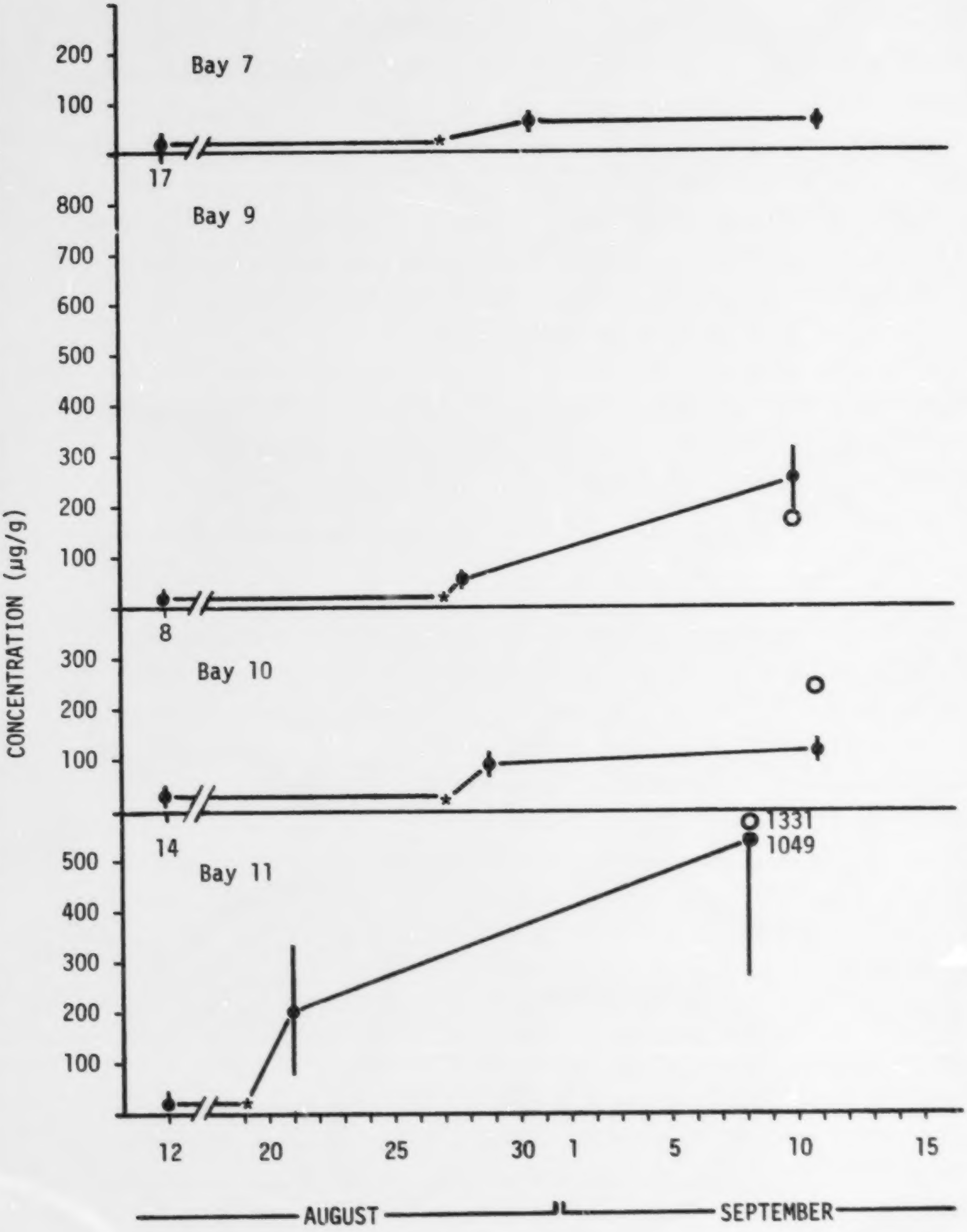


Figure 3.5 Trends in oil concentration in Strongylocentrotus droebachiensis, expressed as crude oil equivalents (µg/g dry weight), by UV/F (*, day of spill; ○, 5m trap sample)

A more detailed examination of the individual sampling stations is presented in Figures 3.6 to 3.9. In pre-spill urchins, the station samples from the 7 m depth stratum varied by as much as a factor of four. An overall average of pre-spill tissue hydrocarbons (UV/F) from all four bays was 17.4 ug/g. The first post-spill samples were more consistent, except from Bay 11 where the values ranged from 20 to 596 ug/g among the 5 stations. Data from the second post-spill sample showed a similar relationship, with only Bay 11 being greatly variable, ranging from 174 to 2453 ug/g. Stations one and four were consistently higher in the post-spill samples of urchins from Bay 11. The urchin trap samples from 5m, examined at the second post-spill time period were the same as those from the 7m depth stratum in Bays 9 and 11, and somewhat higher in the case of Bay 10 (Figures 3.6, 3.7 and 3.9).

3.2.1.2 GC^2/FID

Very few of the urchin samples pooled for each 7m depth stratum contained quantifiable amounts of alkanes (>50 ppm oil equivalents) characteristic of Lagomedio crude oil (Figures 3.10 to 3.13). The urchin samples at Bay 11 (7m, Site No. 1 and 4) which were found to have high fluorescence and PAH content were also found to contain alkanes at both the first and second time points post-spill (Figures 3.14 and 3.15). The urchin trap samples at 5m from Bays 9, 10 and 11 also contained n-alkanes, the levels again being much higher in samples from Bay 11 (Figure 3.16). The results, calculated as outlined in the methods section, are presented in Table 3.1, and the abundance of the n-alkanes from C16 to C26 relative to C24 is shown in Figure 3.17.

Site No. 1 in Bay 11 was the most highly contaminated, with tissue levels in excess of 2,000 ug/g at the second time point post-spill. The distribution of the n-alkanes and the phytane/n-C18 ratio was also very similar to that in the oil at both time points. At Site No. 4 on the same transect, the distribution of n-alkanes below C23 was significantly different from that in the oil at both time points post-spill. This

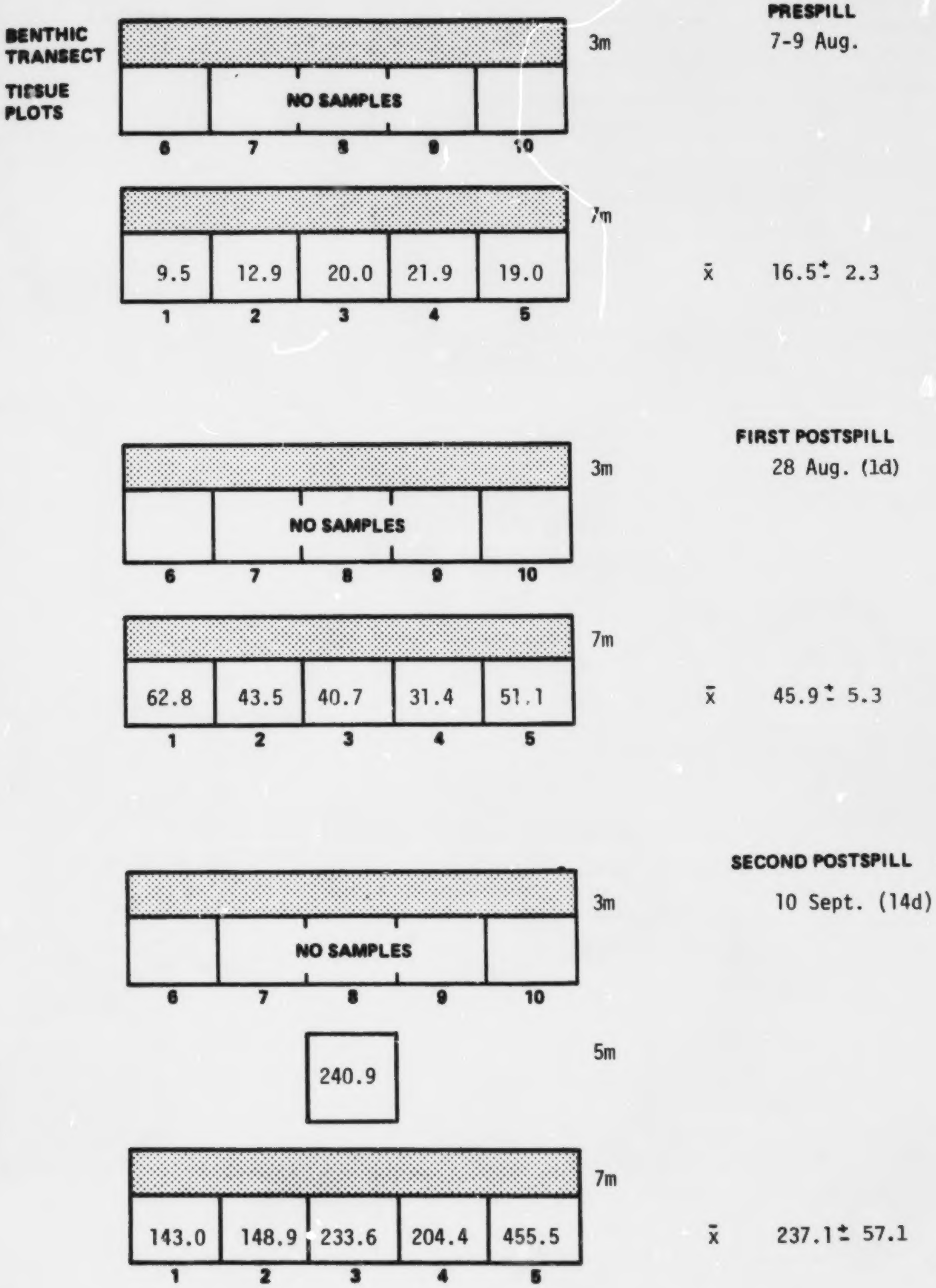

BENTHIC TRANSECT
TISSUE PLOTS
PRESPILL
7-9 Aug.
3m
NO SAMPLES
6
7
8
9
10
7m
9.5
12.9
20.0
21.9
19.0
1
2
3
4
5
x̄ 16.5 ± 2.3
FIRST POSTSPILL
28 Aug. (1d)
3m
NO SAMPLES
6
7
8
9
10
7m
62.8
43.5
40.7
31.4
51.1
1
2
3
4
5
x̄ 45.9 ± 5.3
SECOND POSTSPILL
10 Sept. (14d)
3m
NO SAMPLES
6
7
8
9
10
5m
240.9
7m
143.0
148.9
233.6
204.4
455.5
1
2
3
4
5
x̄ 237.1 ± 57.1

Figure 3.6 Concentrations of oil in Strongylocentrotus droebachiensis, Bay 9, by UV/F (µg/g dry weight), ± S.E.

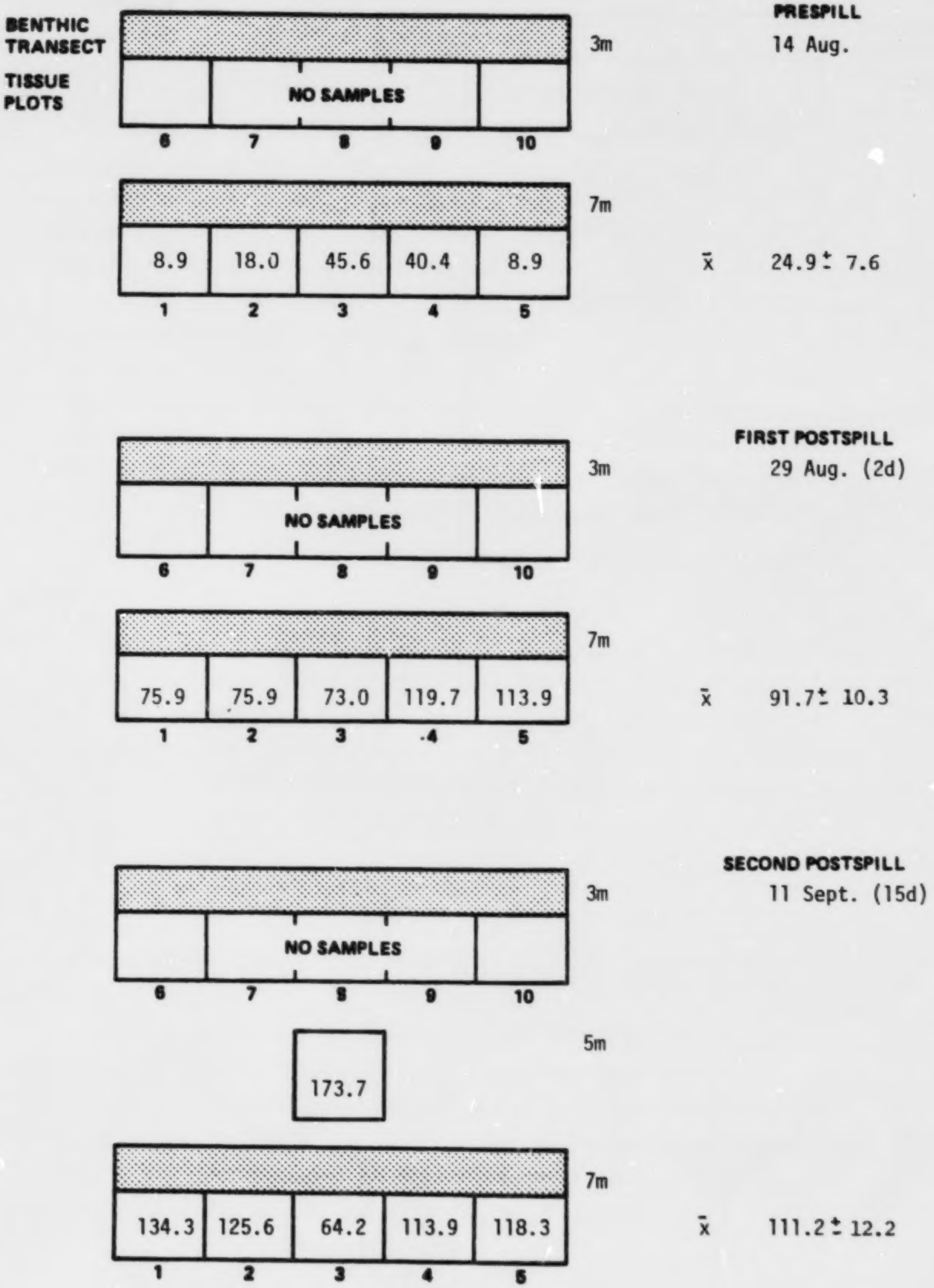

BENTHIC TRANSECT
TISSUE PLOTS
PRESPILL
14 Aug.
3m
NO SAMPLES
6
7
8
9
10
7m
8.9
18.0
45.6
40.4
8.9
1
2
3
4
5
x̄ 24.9 ± 7.6
FIRST POSTSPILL
29 Aug. (2d)
3m
NO SAMPLES
6
7
8
9
10
7m
75.9
75.9
73.0
119.7
113.9
1
2
3
4
5
x̄ 91.7 ± 10.3
SECOND POSTSPILL
11 Sept. (15d)
3m
NO SAMPLES
6
7
8
9
10
5m
173.7
7m
134.3
125.6
64.2
113.9
118.3
1
2
3
4
5
x̄ 111.2 ± 12.2

Figure 3.7 Concentrations of oil in Strongylocentrotus droebachiensis, Bay 10, by UV/F (μg/g dry weight), ± S.E.

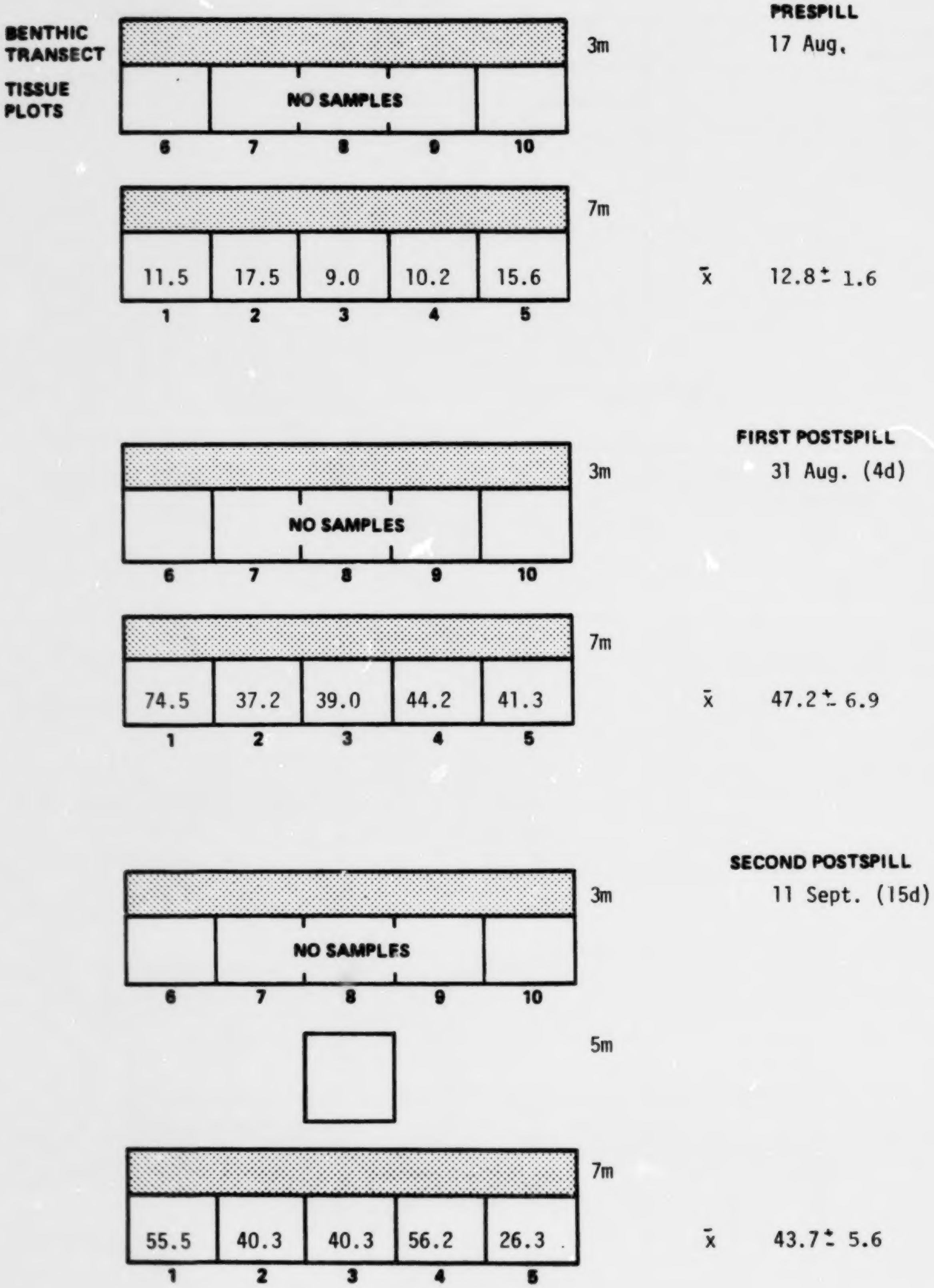

BENTHIC TRANSECT
TISSUE PLOTS
PRESPILL
17 Aug.
3m
NO SAMPLES
6
7
8
9
10
7m
11.5
17.5
9.0
10.2
15.6
1
2
3
4
5
x̄ 12.8 ± 1.6
FIRST POSTSPILL
31 Aug. (4d)
3m
NO SAMPLES
6
7
8
9
10
7m
74.5
37.2
39.0
44.2
41.3
1
2
3
4
5
x̄ 47.2 ± 6.9
SECOND POSTSPILL
11 Sept. (15d)
3m
NO SAMPLES
6
7
8
9
10
5m
7m
55.5
40.3
40.3
56.2
26.3
1
2
3
4
5
x̄ 43.7 ± 5.6

Figure 3.8 Concentrations of oil in *Strongylocentrotus droebachiensis*, Bay 7, by UV/F (μg/g dry weight), ± S.E.

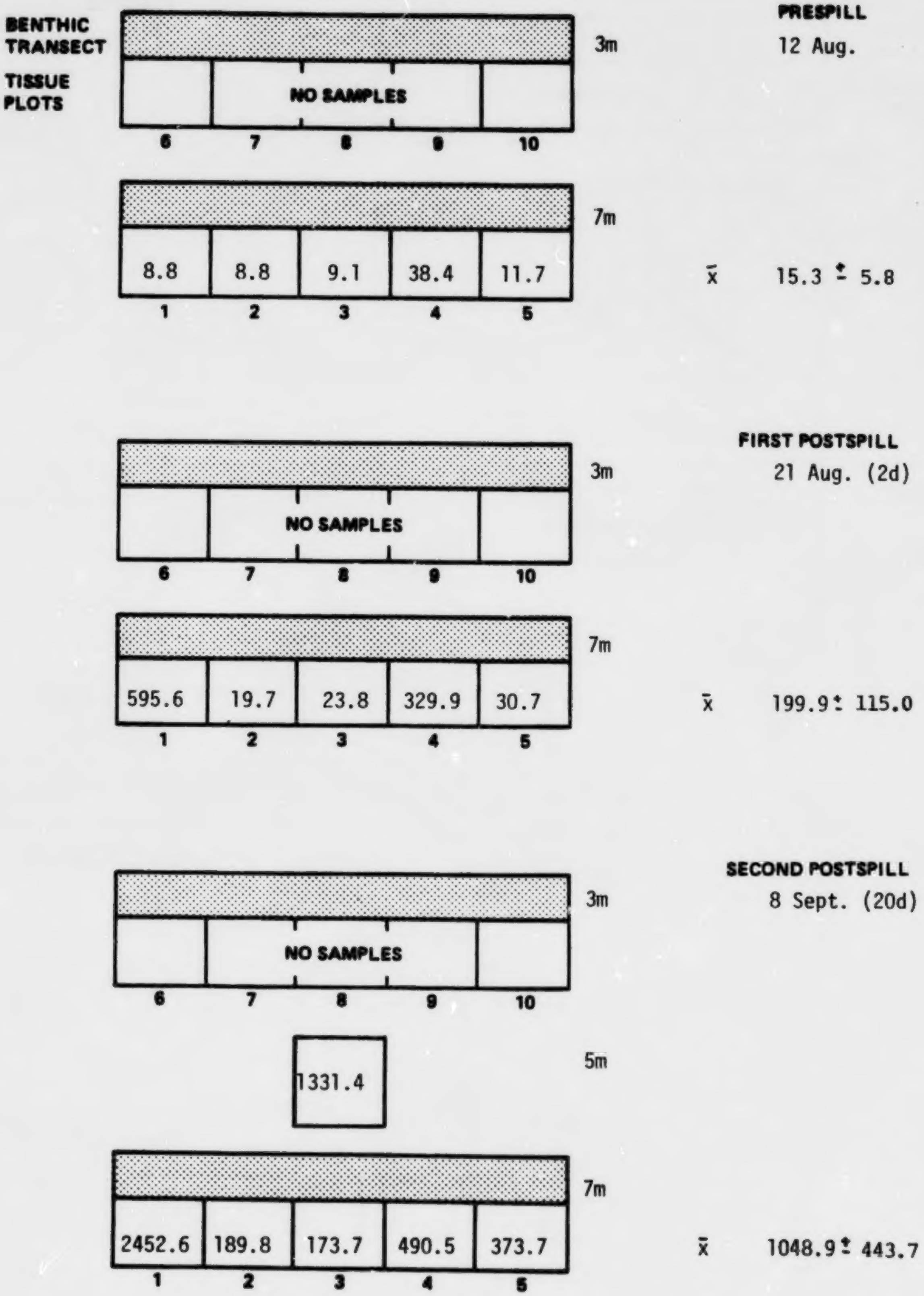

BENTHIC TRANSECT
TISSUE PLOTS
PRESPILL
12 Aug.
3m
NO SAMPLES
6
7
8
9
10
7m
8.8
8.8
9.1
38.4
11.7
1
2
3
4
5
x̄ 15.3 ± 5.8
FIRST POSTSPILL
21 Aug. (2d)
3m
NO SAMPLES
6
7
8
9
10
7m
595.6
19.7
23.8
329.9
30.7
1
2
3
4
5
x̄ 199.9 ± 115.0
SECOND POSTSPILL
8 Sept. (20d)
3m
NO SAMPLES
6
7
8
9
10
5m
1331.4
7m
2452.6
189.8
173.7
490.5
373.7
1
2
3
4
5
x̄ 1048.9 ± 443.7

Figure 3.9 Concentrations of oil in Strongylocentrotus droebachiensis, Bay 11, by UV/F (µg/g dry weight), ± S.E.

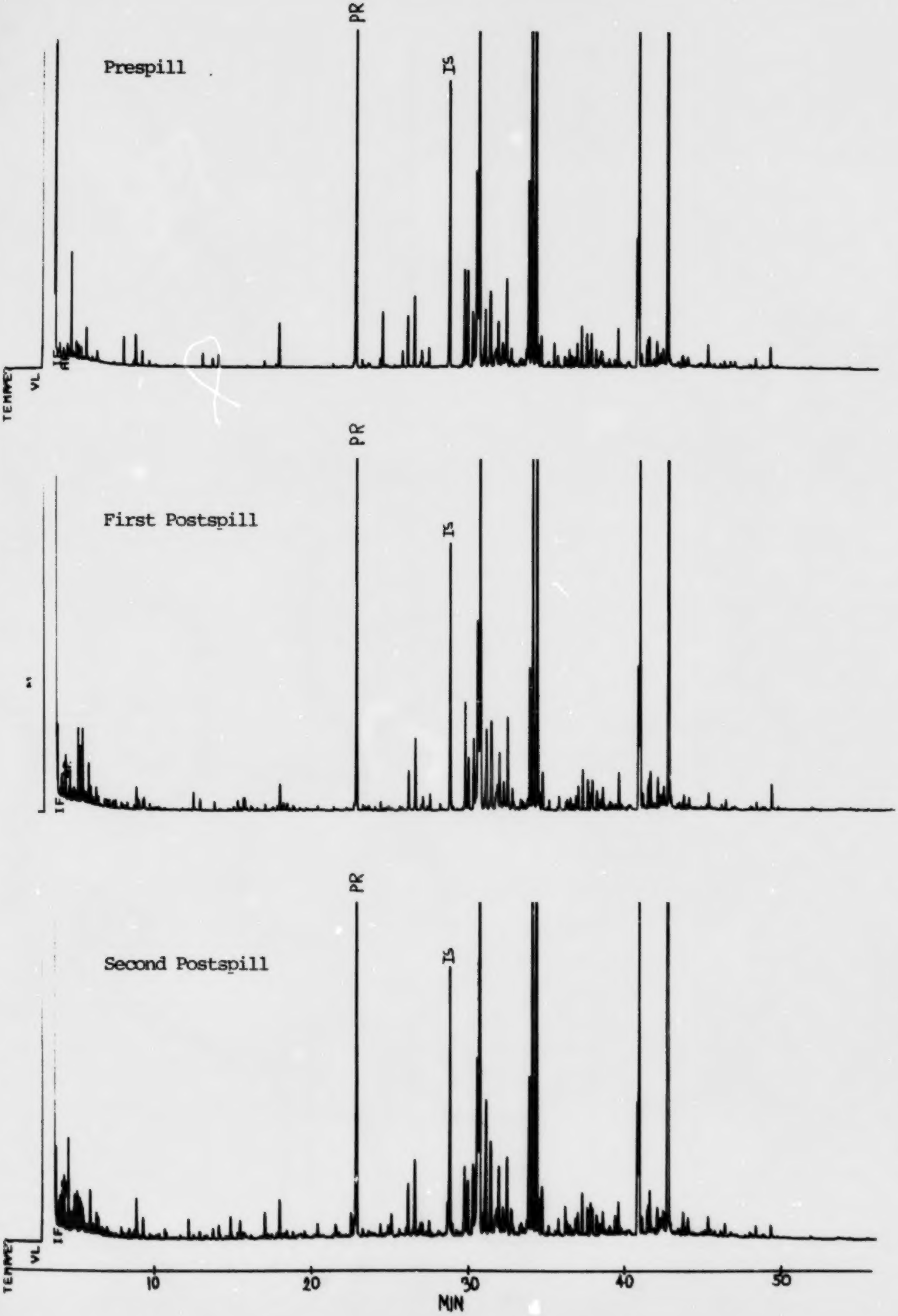


Figure 3.10 GC^2/FID chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 9

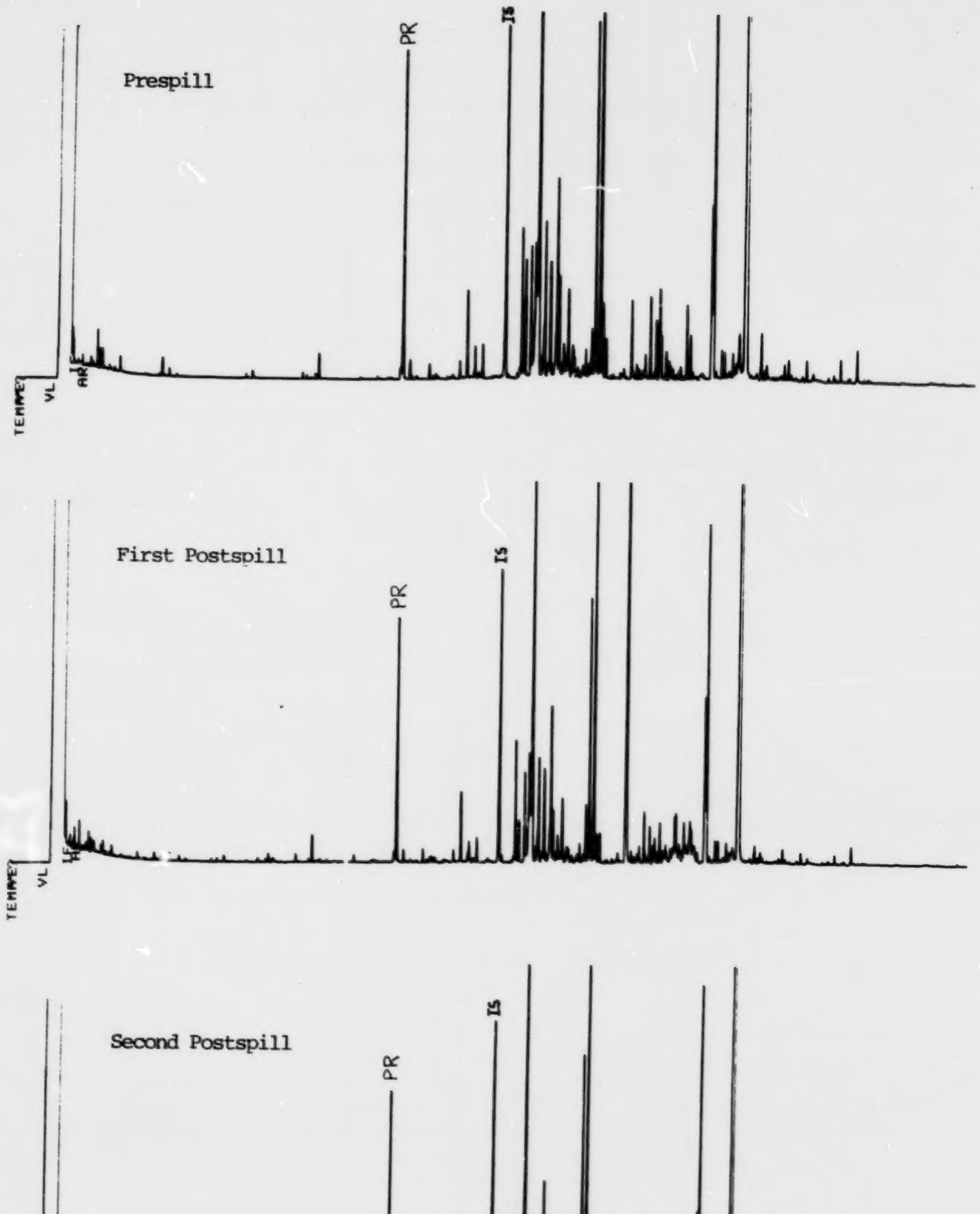


Figure 3.11 GC^2/FID chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 10

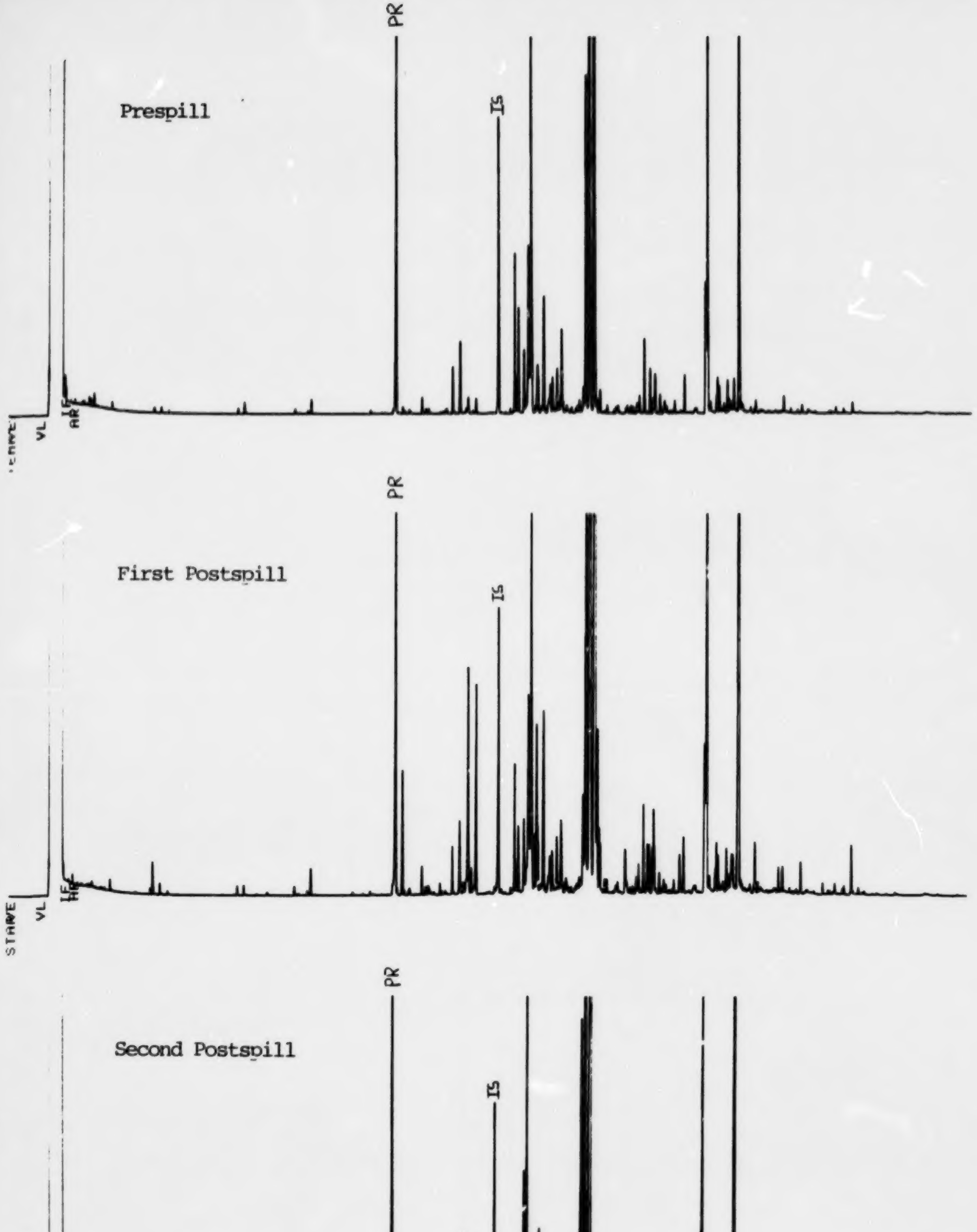


Figure 3.12 GC^2/FID chromatograms of Strongylocentrotus droebachiensis tissues from Bay 7

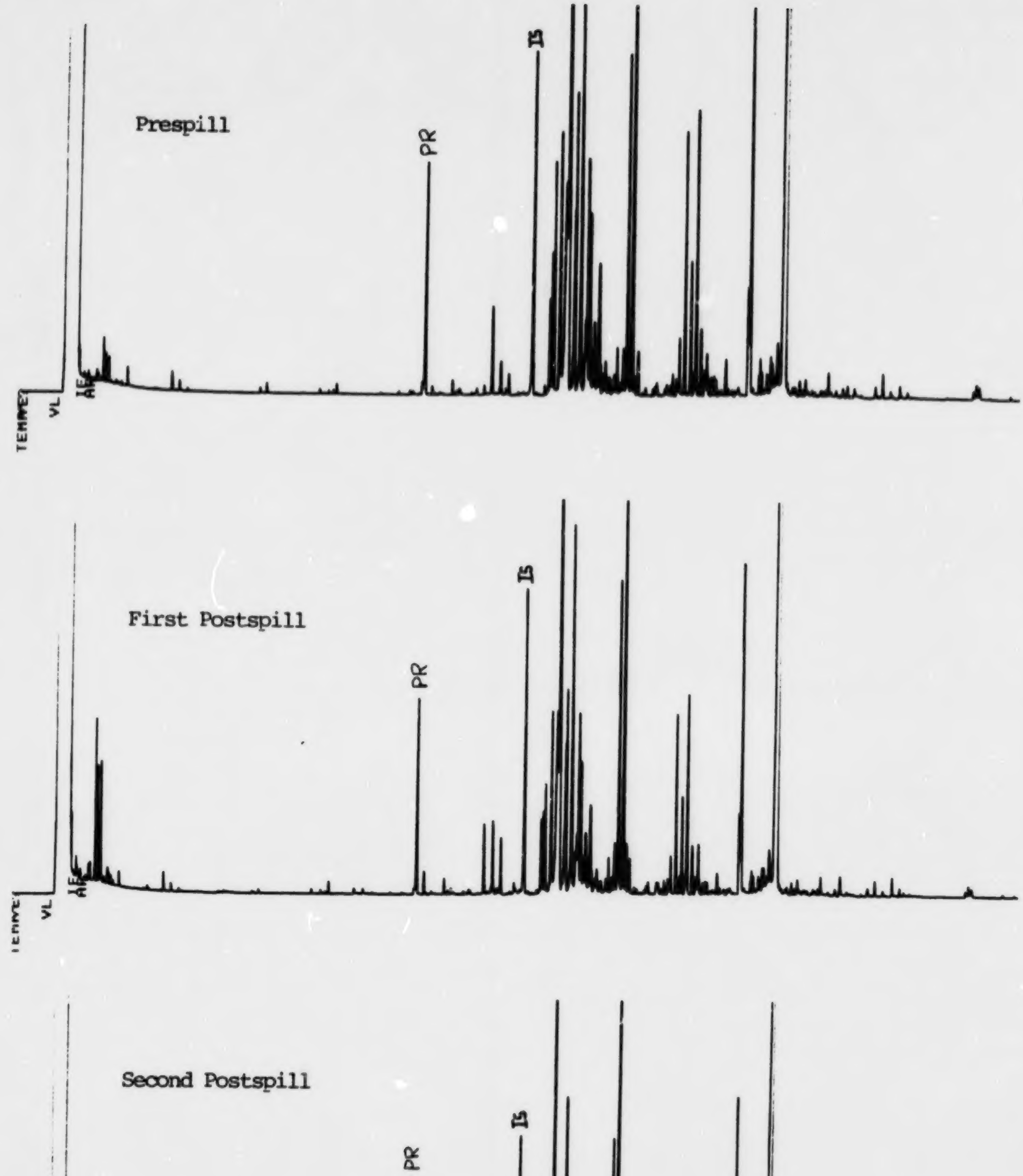


Figure 3.13 GC^2/FID chromatograms of Strongylocentrotus droebachiensis tissues from Bay 11, sampling stations 2,3,and 5 combined

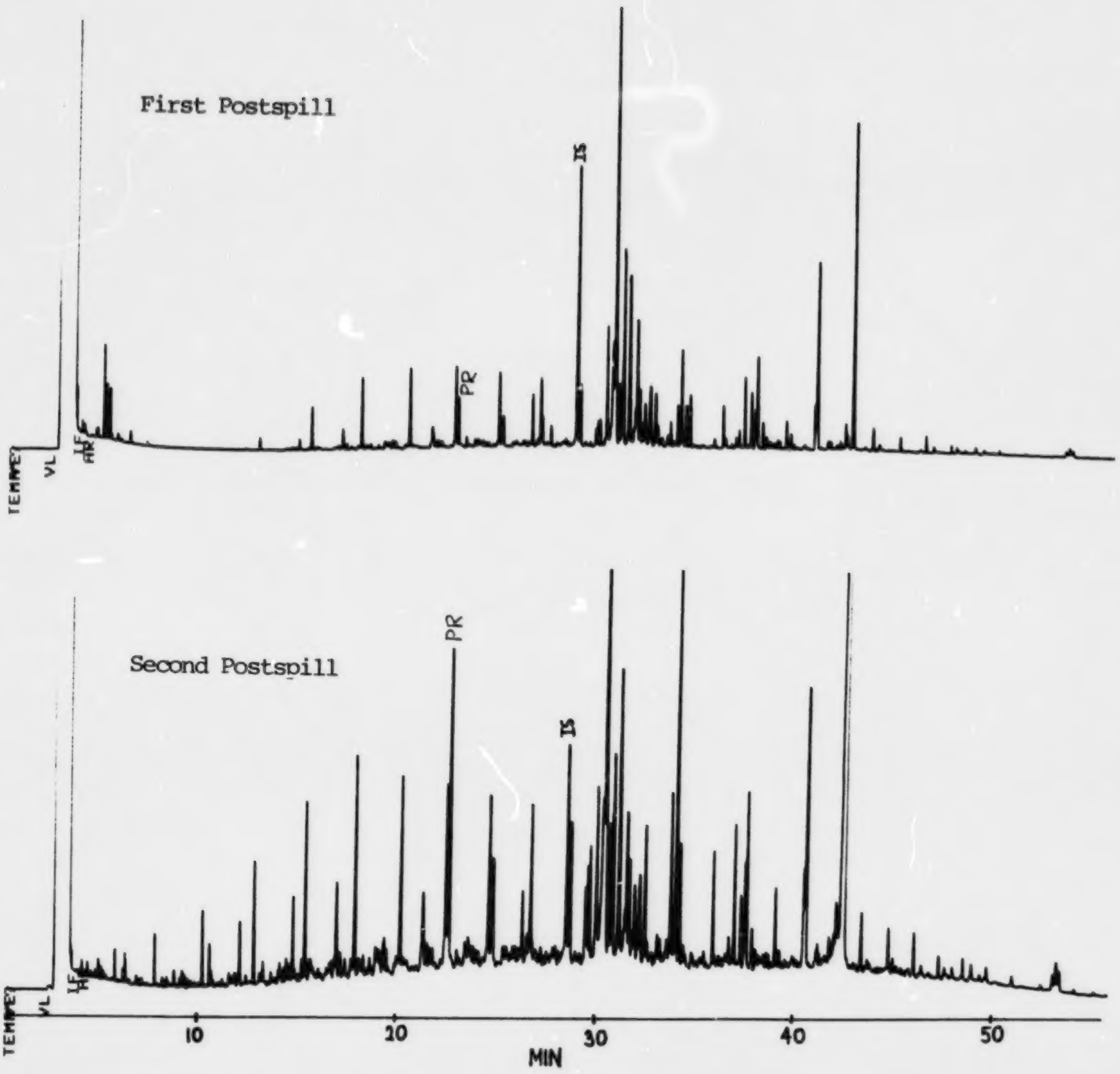


Figure 3.14 GC^2/FID chromatograms of Strongylocentrotus droebachiensis tissues from Bay 11, sampling station 1

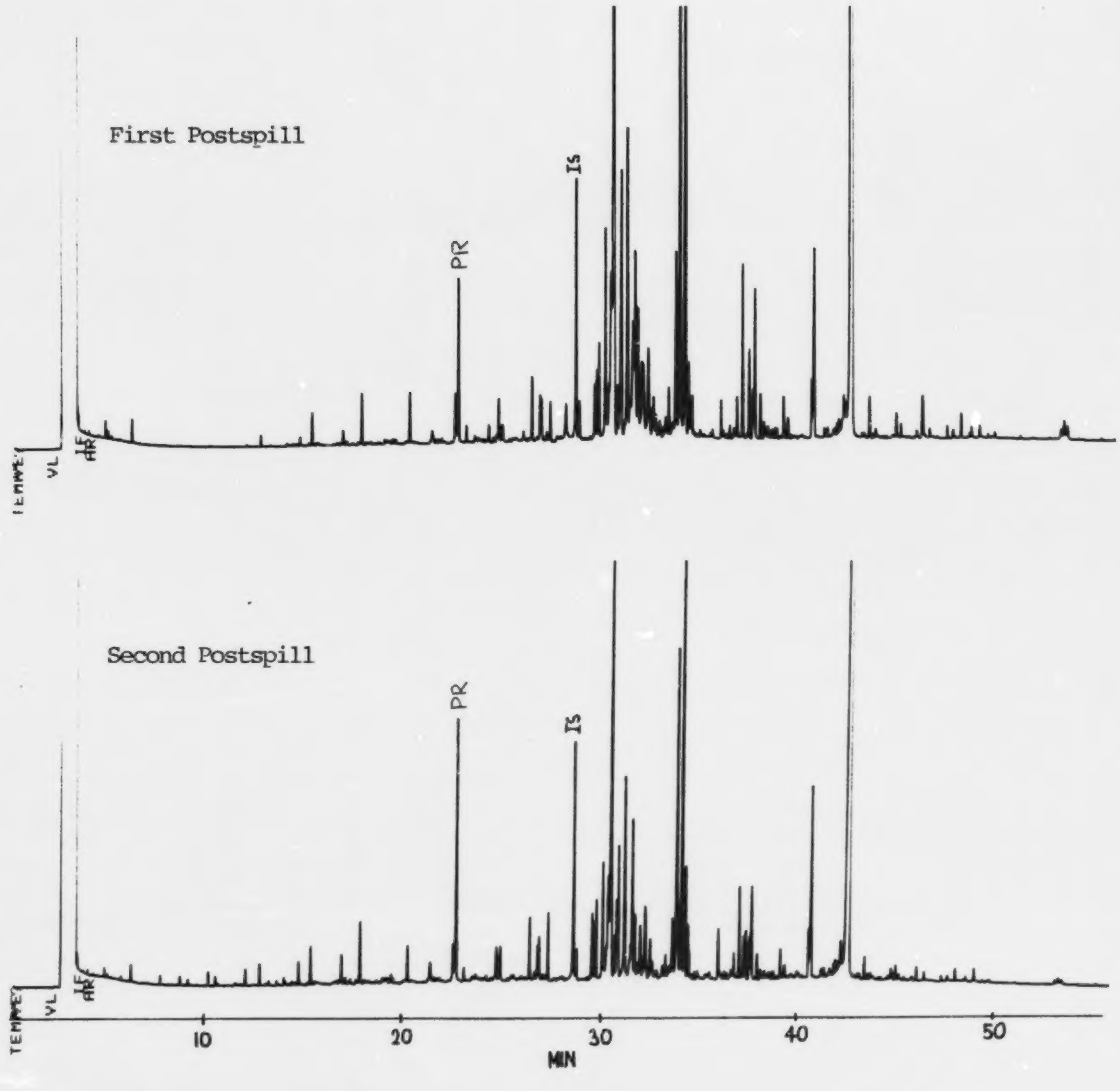


Figure 3.15 GC^2/FID chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 11, sampling station 4

Table 3.1 n - Alkane levels in Strongylocentrotus droebachiensis tissue[1] (ug crude oil/g dry weight) based on sum of peak height of C16-C26 n-alkanes, compared to oil std. by FID

Site	Time Post-Spill	No. in Pool	n-alkane ug/g [2]	Pristane/ Phytane	Phytane/ n-C_{18}
Bay 11 7m, No. 1	1st	7	838	1.6	0.41
	2nd	10	2,349	3.1	0.63
Bay 11 7m, No. 4	1st	8	645	10.1	0.38
	2nd	10	519	7.4	1.00
Bay 11 Urchin trap	2nd	10	1,890	2.2	0.64
Bay 9 Urchin trap	2nd	9	243[2]	15.0	3.10
Bay 10 Urchin trap	2nd	9	121[2]	12.3	2.10
Crude Oil			-	0.9	0.40

[1] Percent water in soft tissue 86.5 ± 1.0

[2] Under 300 ug/g n-alkane levels quantitation is less reliable Detection limit is 100 ug/g.

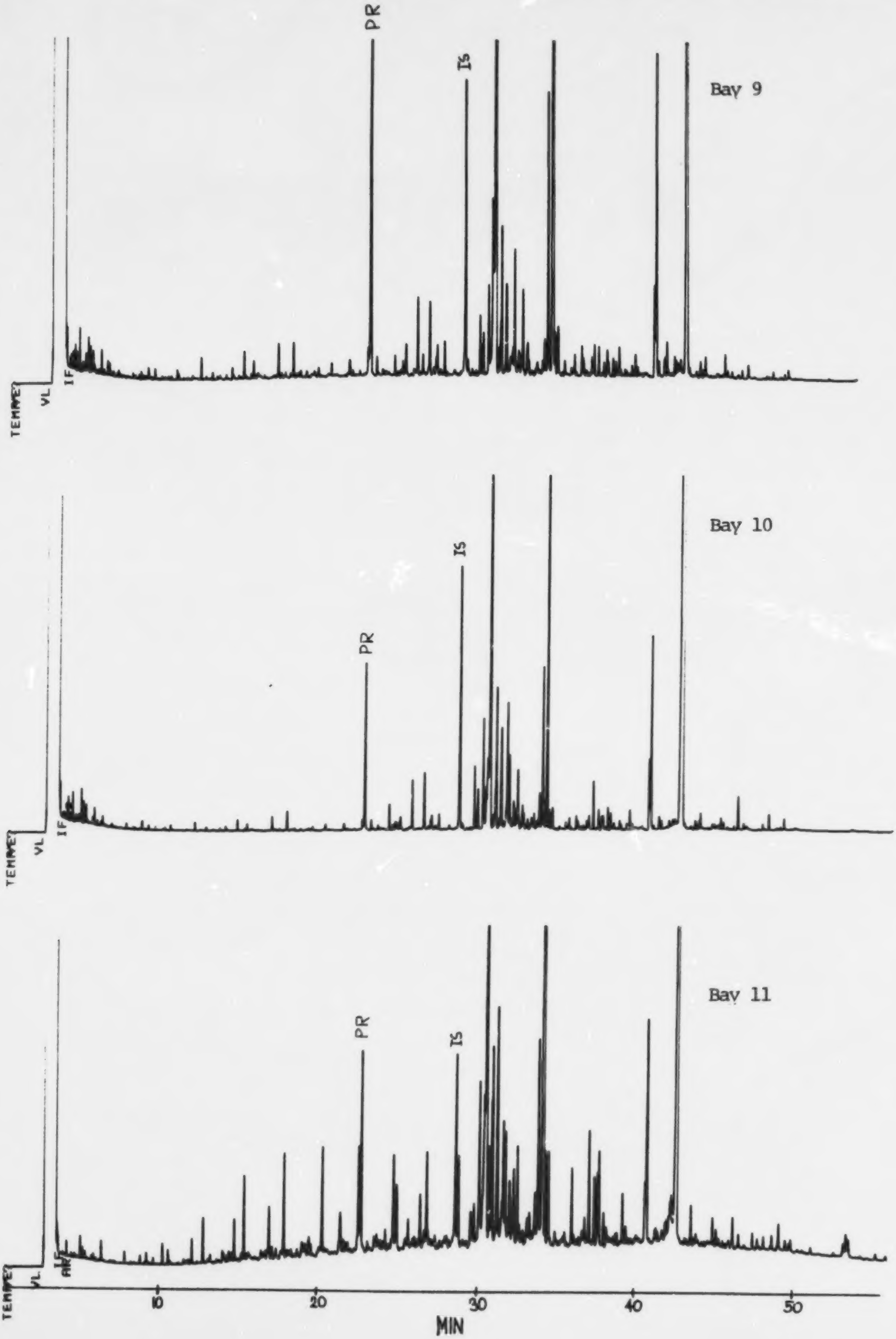


Figure 3.16 GC^2/FID chromatograms of Strongylocentrotus droebachiensis tissues from urchin traps at 5 m depth in three bays

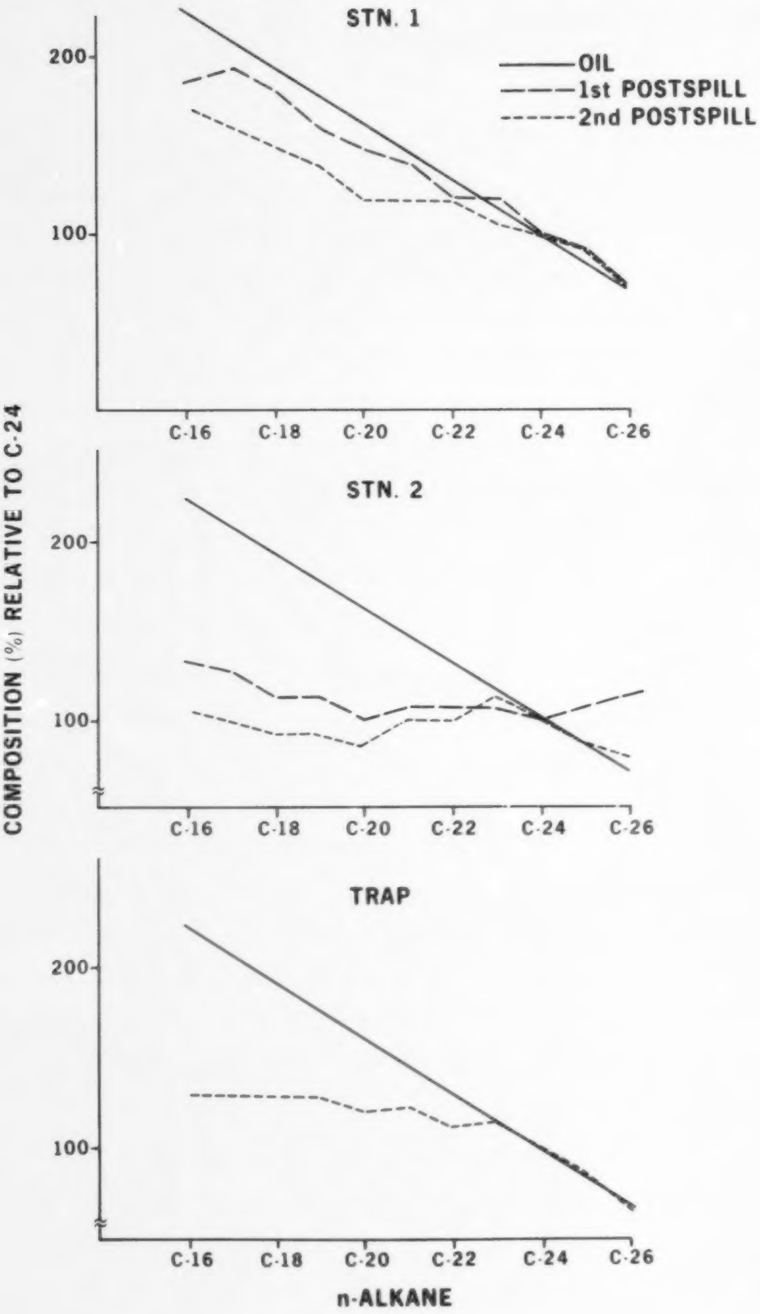


Figure 3.17 Relative n-alkane composition of Strongylocentrotus droebachiensis tissues from Bay 11

pattern existed also for the urchin trap sample from Bay 11 (Figure 3.18). The phytane/n-C18 ratio was higher than that in the oil at the second time point post-spill, especially in the Bay 9 and 10 urchin trap samples. Pristane and phytane levels in urchin samples from all four bays, pooled by transect, are presented in Table 3.2.

3.2.1.3 GC^2/SIM

The aromatic hydrocarbon profiles of urchin tissues from Bays 9, 10 and 11 indicate a proportional emphasis on the lower molecular weight hydrocarbons in the first post-spill samples (Figures 3.19 to 3.21). This is especially clear in the Bay 9 first post-spill urchin sample which showed naphthalene and alkyl benzene levels over 2,000 ng/g. The data are summarized in Figures 3.22 and 3.23. No such relative enhancements of the low molecular weight aromatics was noted for the first post-spill sample from Bay 11. While relatively low aromatic concentrations were found in the pooled samples from Stations 2, 3 and 5 (Figures 3.23 and 3.24), the two individual station samples from this surface-spill bay clearly showed a difference in early uptake patterns as compared to urchins from the dispersed oil bays (Figures 3.25 to 3.27).

Urchin tissues from the second post-spill samples exhibited an enhancement in the relative proportions of larger molecular weight aromatics, as well as a shift to a greater degree of methylation within an aromatic group (Figures 3.19 to 3.27). Levels over 2000 ng/g are recorded in urchin tissues from dispersed oil spill exposures; especially C3- and C4-naphthalene, C2-phenanthrene, and C2- and C3- dibenzthiophene. The second post-spill samples for Bay 11 tissues similarly showed a relative predominance of larger molecular weight, polymethylated aromatics. Station 1 and 4 samples from Bay 11 illustrated this to the greatest degree, with individual selected PAH's from Station 1 showing concentration values over 3,000 ng/g (Figure 3.27).

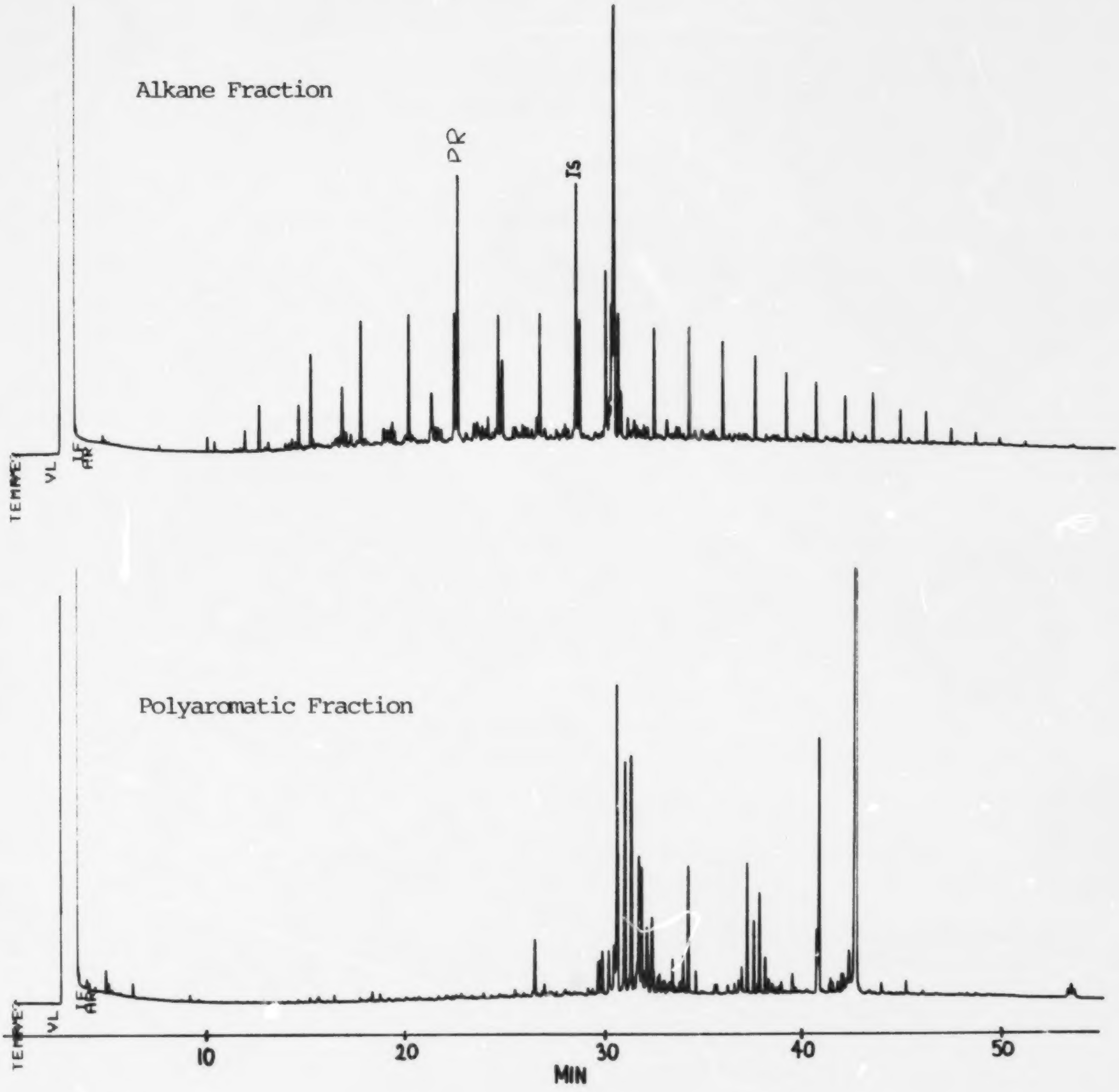


Figure 3.18 GC^2/FID chromatograms (alkanes and polyaromatic hydrocarbons separated) of *Strongylocentrotus droebachiensis* tissues from the urchin trap at 5 m in Bay 11

Table 3.2 Pristane and phytane levels (ug/g) dry weight)[1] and ratios in Strongylocentrotus droebachiensis

Site	Time Re.Spill	No. in Pool		Pristane	Phytane	Pristane-Phytane Ratio
		Stn.	Indiv.			
Bay 9-7m	pre	5	61	22.9	<0.20	>115
	1st	4	50	24.1	<0.20	>121
	2nd	5	55	28.7	1.36	21
Bay 10-7m	pre	5	44	14.4	<0.20	>70
	1st	5	38	11.8	<0.28	>42
	2nd	5	49	11.8	0.64	18
Bay 7-7m	pre	5	45	24.6	<0.20	>123
	1st	5	48	19.4	<0.20	> 97
	2nd	5	49	22.3	0.41	54
Bay 11-7m	pre	4	31	9.6	<0.20	>48
	1st	3	22	9.4	<0.20	>47
	2nd	3	30	11.7	1.23	10

[1] percent water in soft tissue, 86.5 ± 1.0

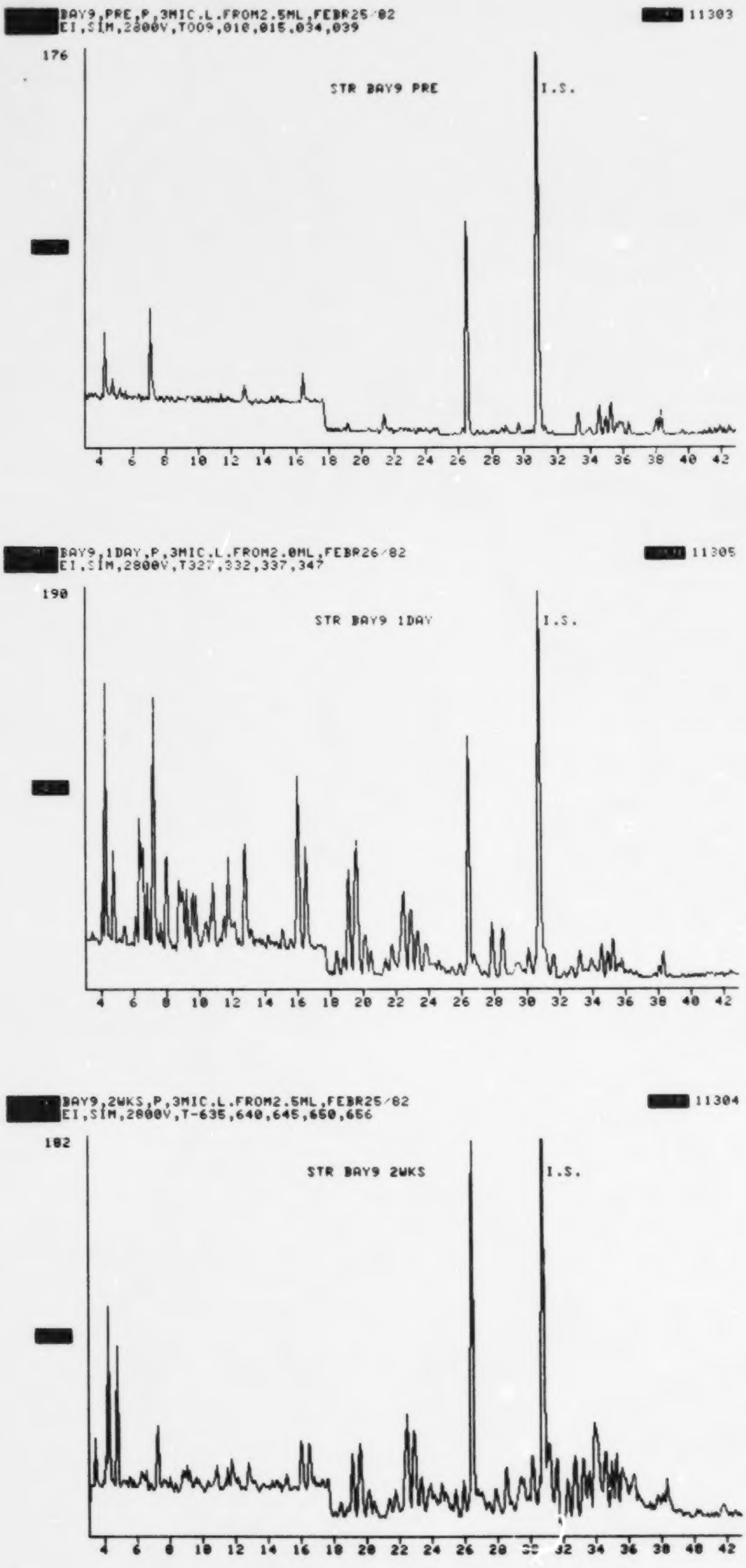


Figure 3.19 GC^2/MS total ion chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 9

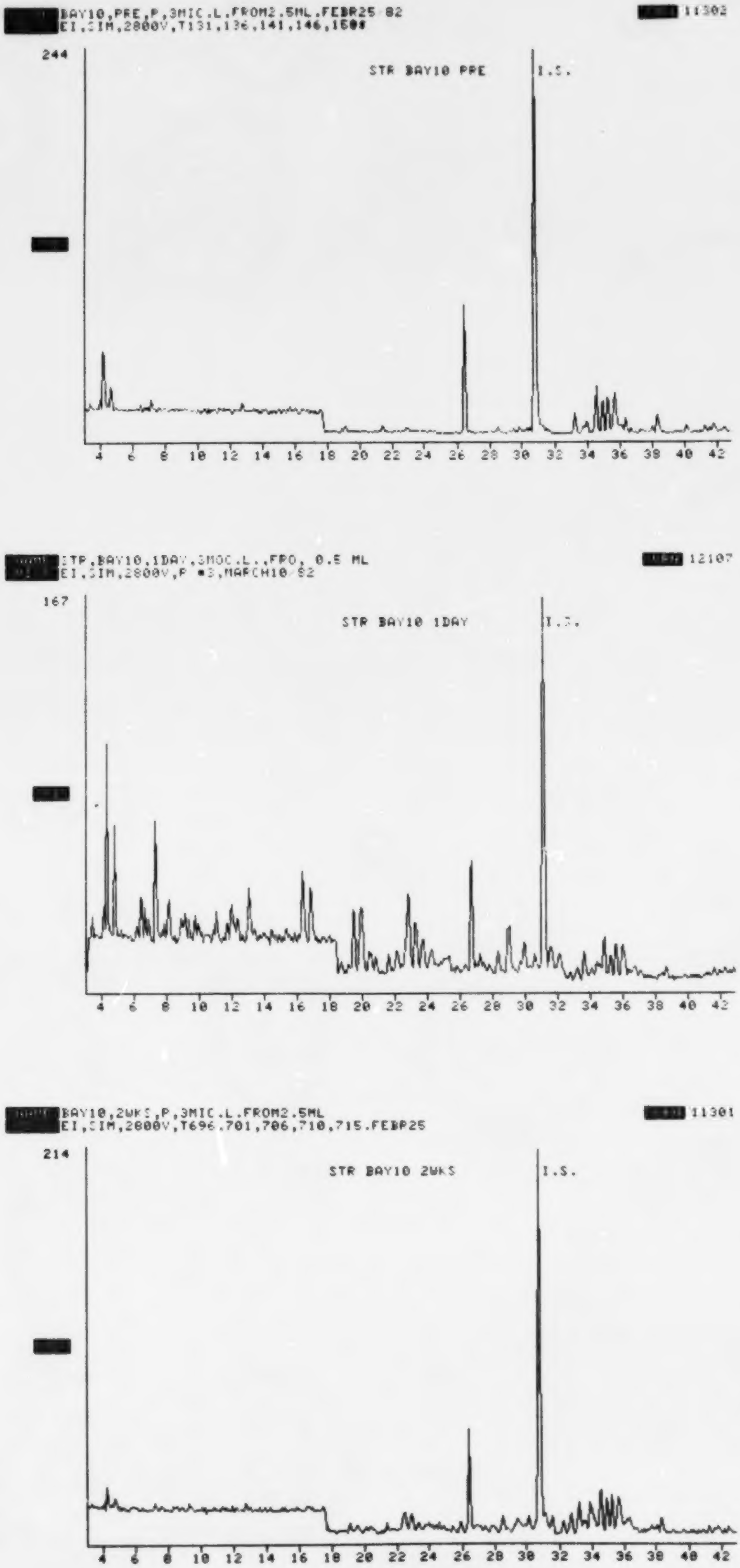


Figure 3.20 GC^2/MS total ion chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 10

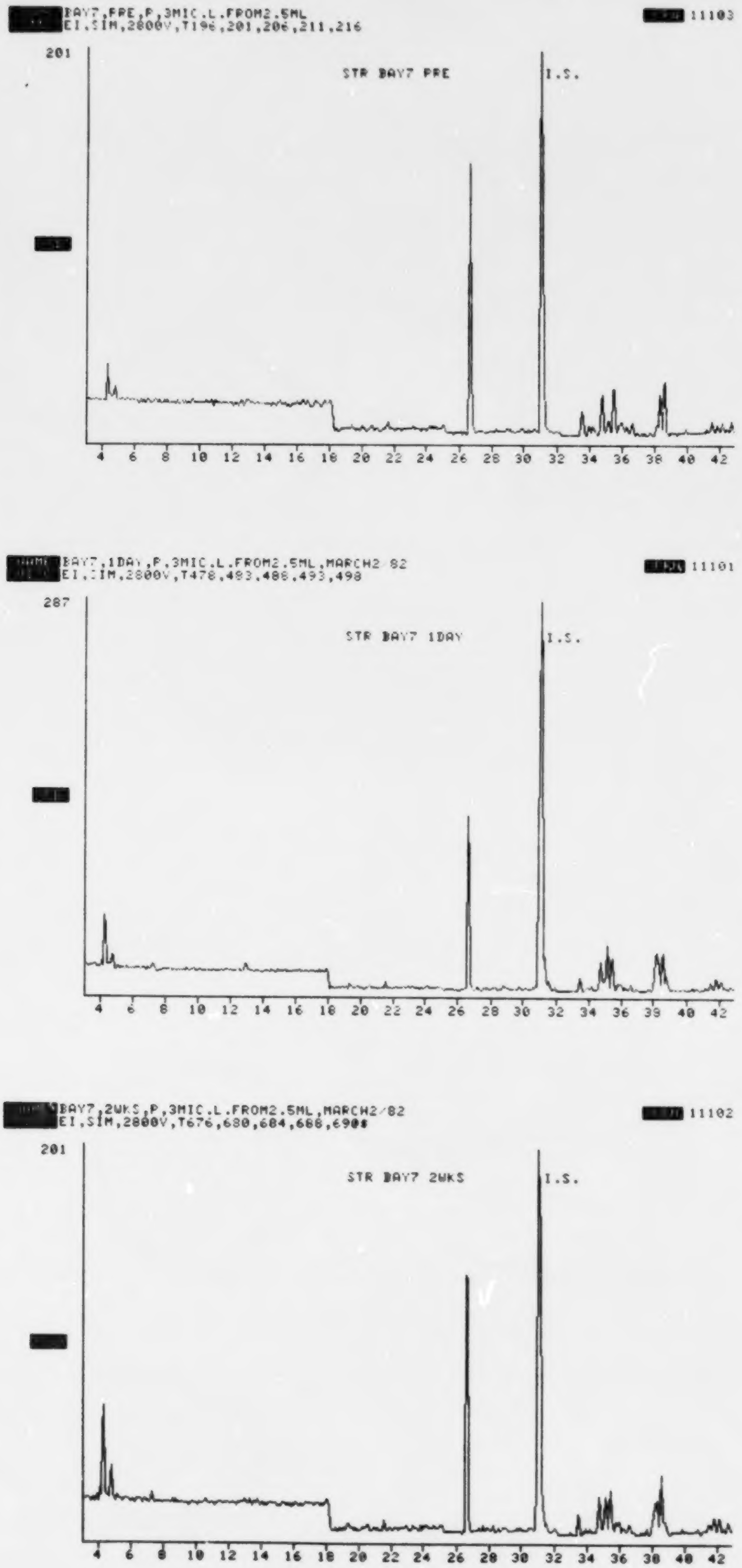


Figure 3.21 GC^2/MS total ion chromatograms of Strongylocentrotus droebachiensis tissues from Bay 7

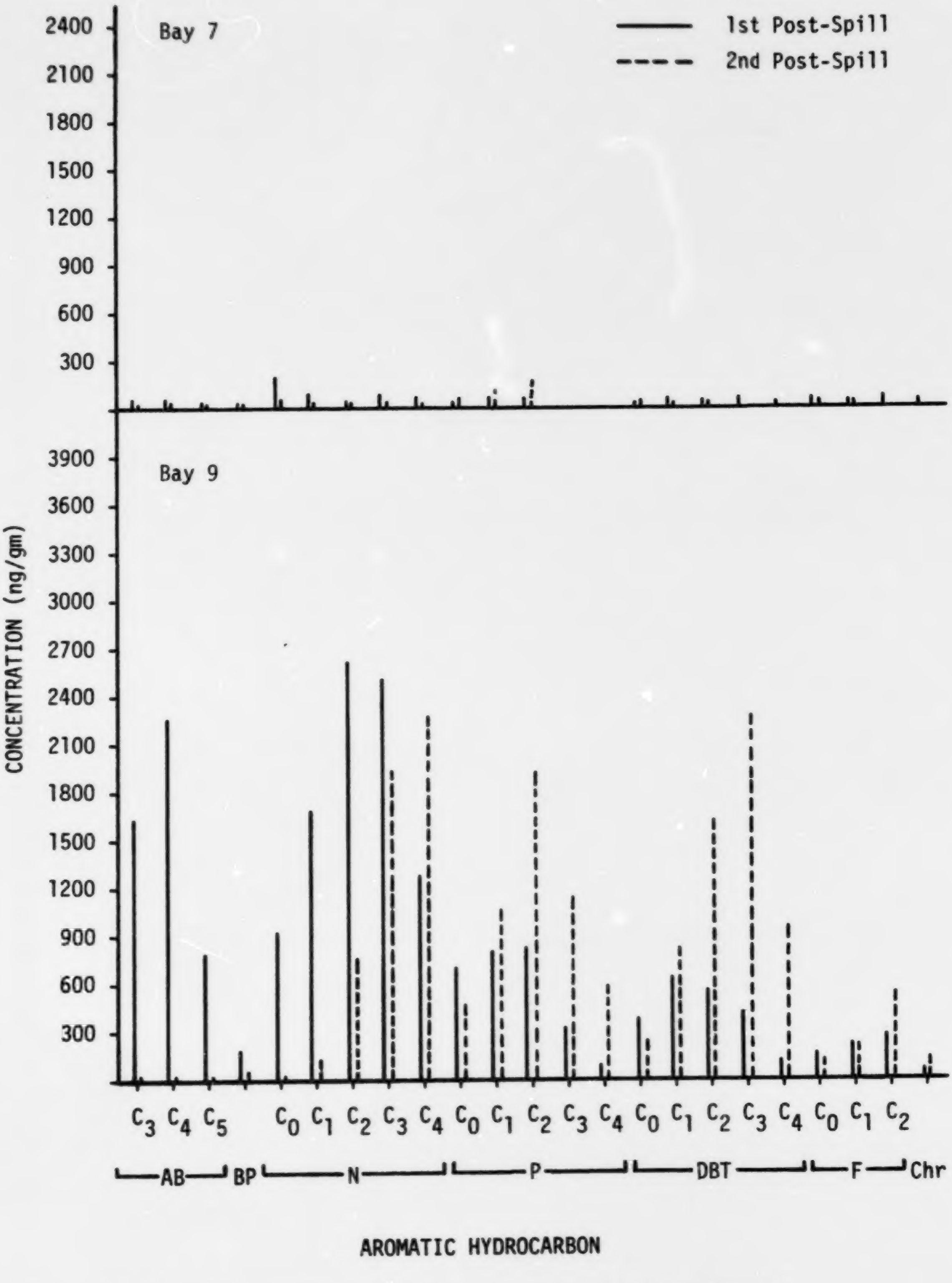


Figure 3.22 Aromatic profiles in *Strongylocentrotus droebachiensis*

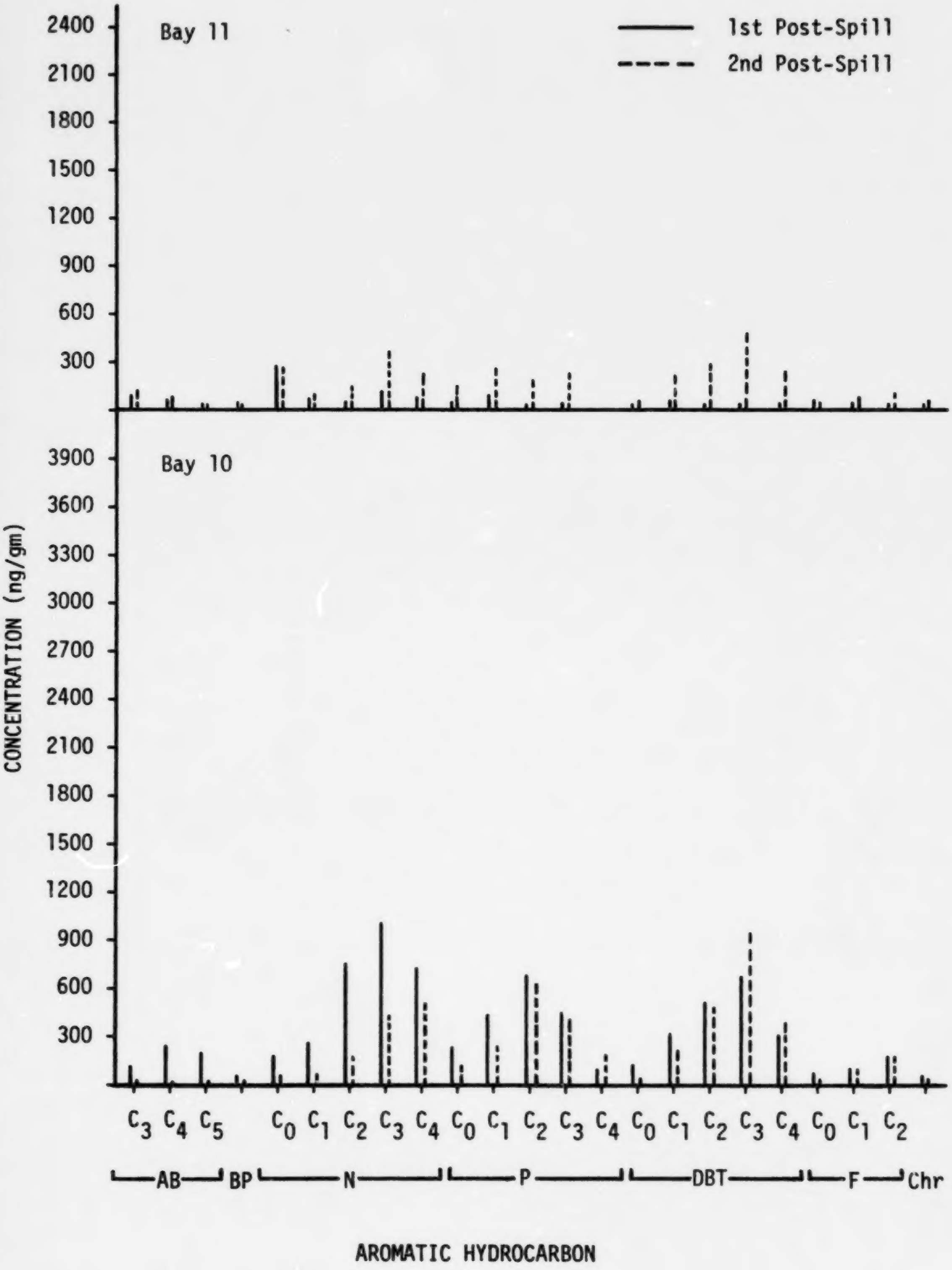


Figure 3.23 Aromatic profiles in Strongylocentrotus droebachiensis

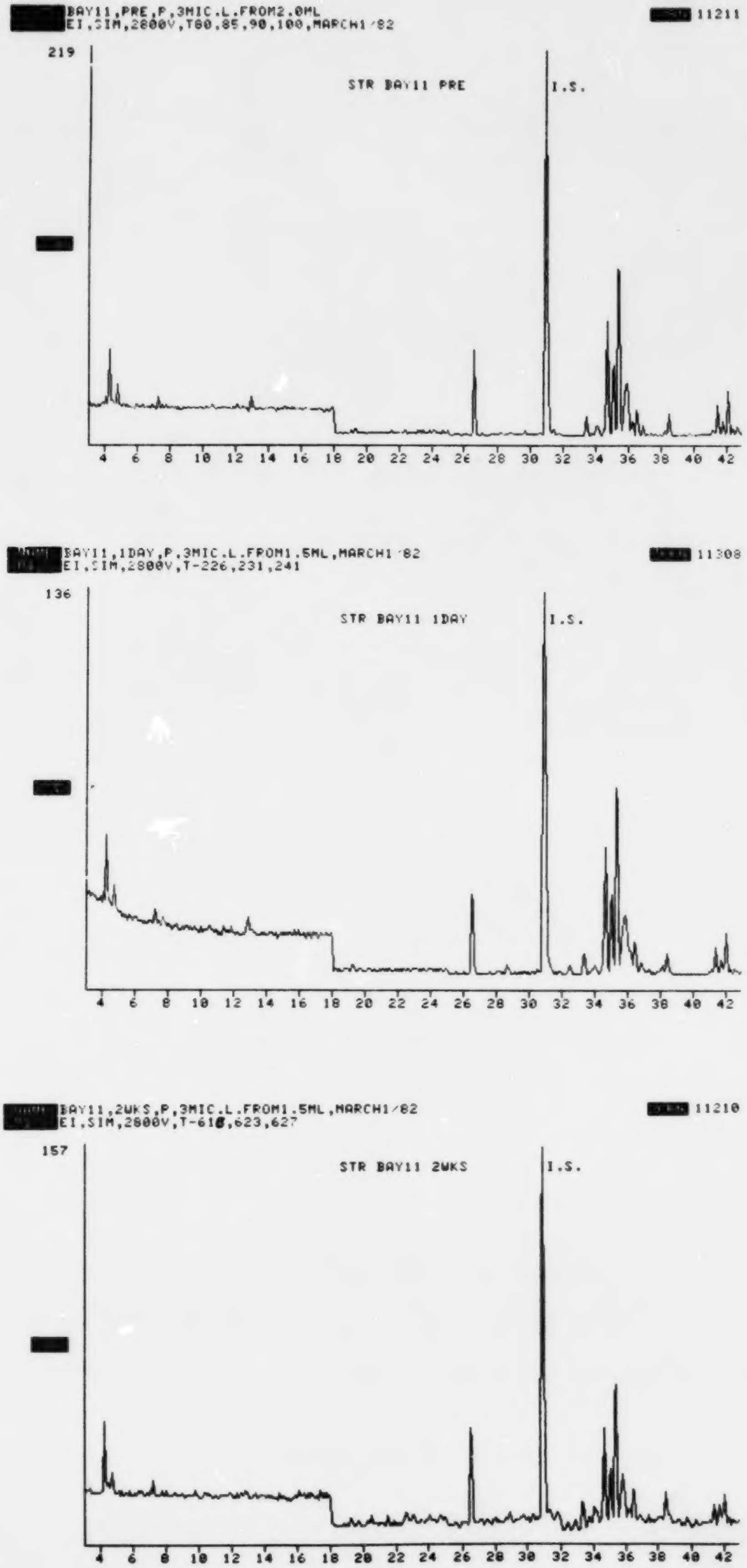


Figure 3.24 GC^2/MS total ion chromatograms of Strongylocentrotus droebachiensis tissues from Bay 11, sampling stations 2,3, and 5 combined

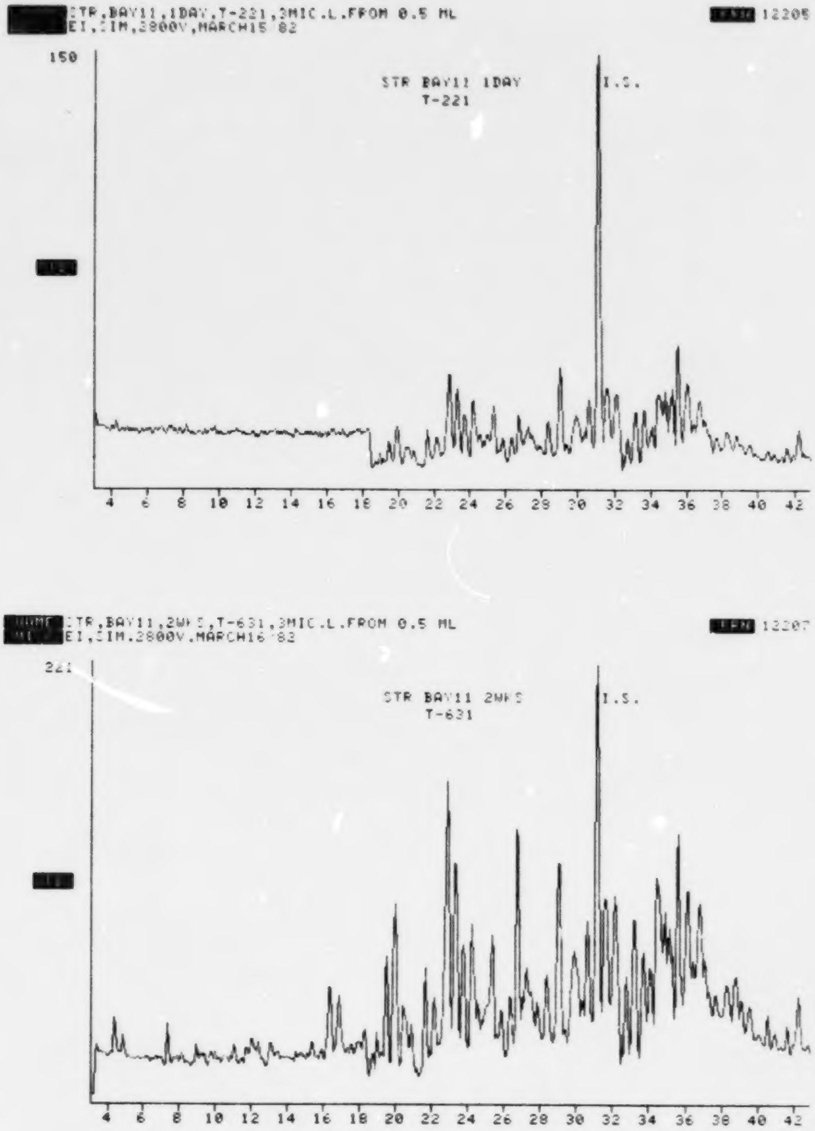


Figure 3.25 GC^2/MS total ion chromatograms of Strongylocentrotus droebachiensis tissues from Bay 11, sampling station 1

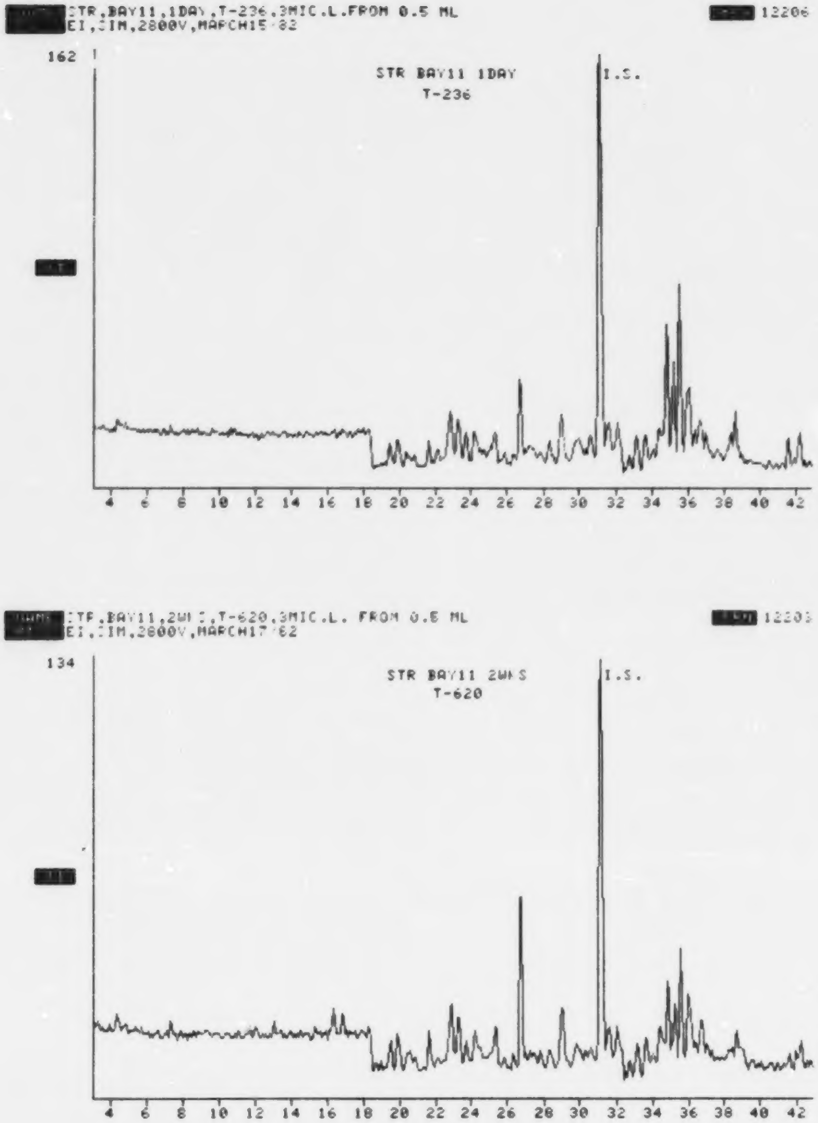


Figure 3.26 GC^2/MS total ion chromatograms of Strongylocentrotus droebachiensis tissues from Bay 11, samplinf station 4

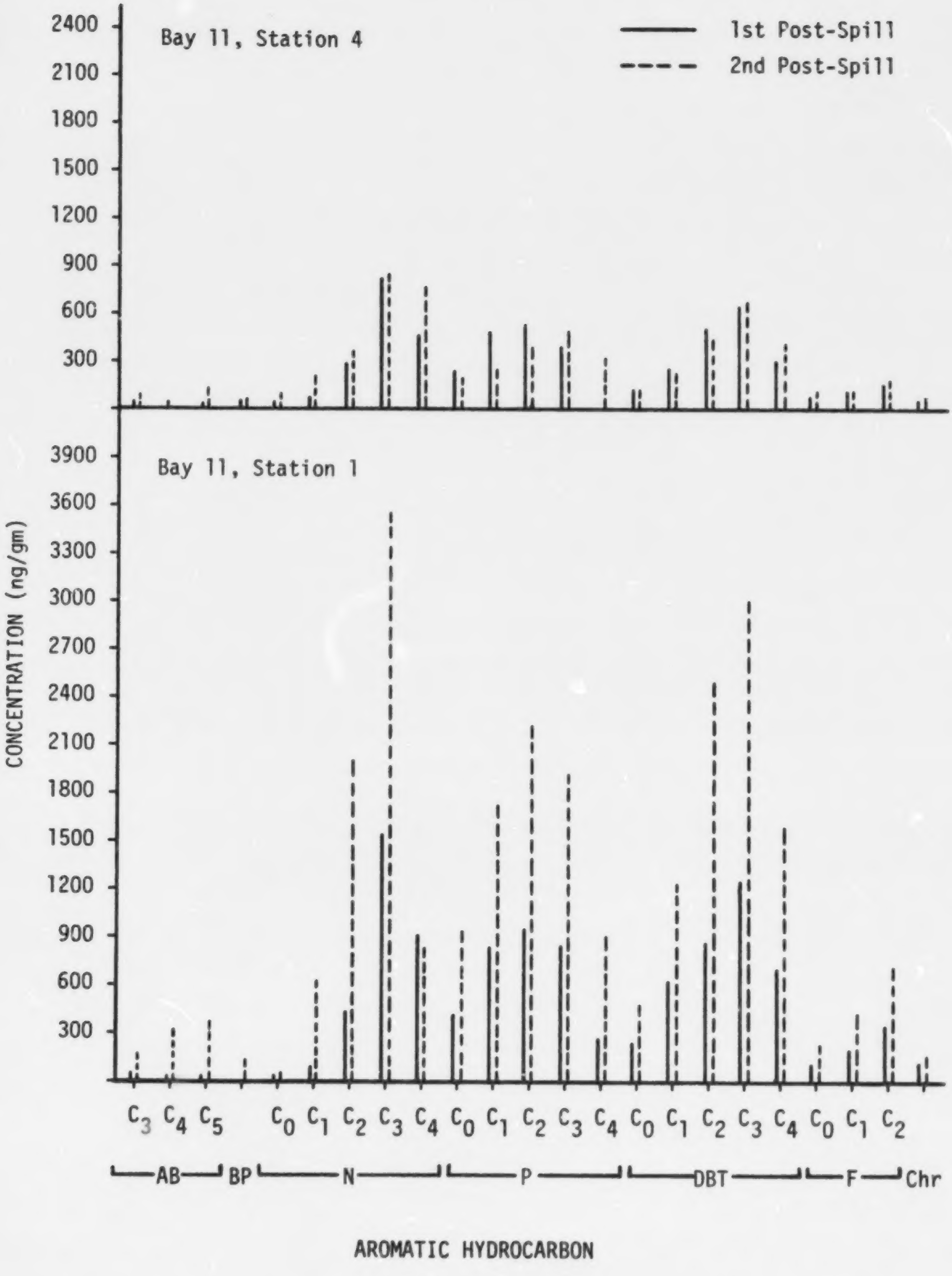


Figure 3.27 Aromatic profiles in Strongylocentrotus droebachiensis

The urchin trap samples from 5m showed PAH characteristics similar to the 7m samples (Figure 3.28). Polymethylated large molecular weight aromatics were enhanced in concentration. A comparison among trap samples from dispersed oil and surface spill conditions shows a marked similarity in relative composition (Figure 3.29).

When total oil concentration in pooled urchin tissues is expressed using the GC^2/SIM data, as related to aromatic levels in the crude oil (Figure 3.30), there are some differences from the concentration values determined by UV/F (Figure 3.5). First post-spill samples in Bays 9, and 10 were markedly higher than when assessed on the basis of UV/F. Second post-spill samples for Bays 9 and 11 were lower on the basis of their aromatic composition, as was the first post-spill sample from Bay 11.

3.2.2 Pectinaria granulosa

3.2.2.1 UV/F

Tissue samples from polychaetes of Bays 9 and 10, pooled for the five stations of each 7m depth stratum, demonstrated an increase in total oil concentrations 1 to 2 days after oiling (Figures 3.31 to 3.33). This increased by two weeks post-spill in Bay 9 polychaetes to nearly 300 ug/g, but remained relatively constant in tissues from Bay 10. No discernible increases were noted 2 to 4 days post-spill in Bay 7 and Bay 11 samples (Figures 3.31, 3.34 and 3.35). Polychaetes from Bay 11 did show an increased body burden in the 2nd post-spill sample. Bay 7 tissues remained clean.

3.2.2.2 GC^2/FID

The amounts of n-alkanes in polychaetes were found to be so low as to be almost unquantifiable. Figures 3.36 to 3.39 demonstrate qualitatively, however, that there was a minimal increase in n-alkanes in post-spill samples.

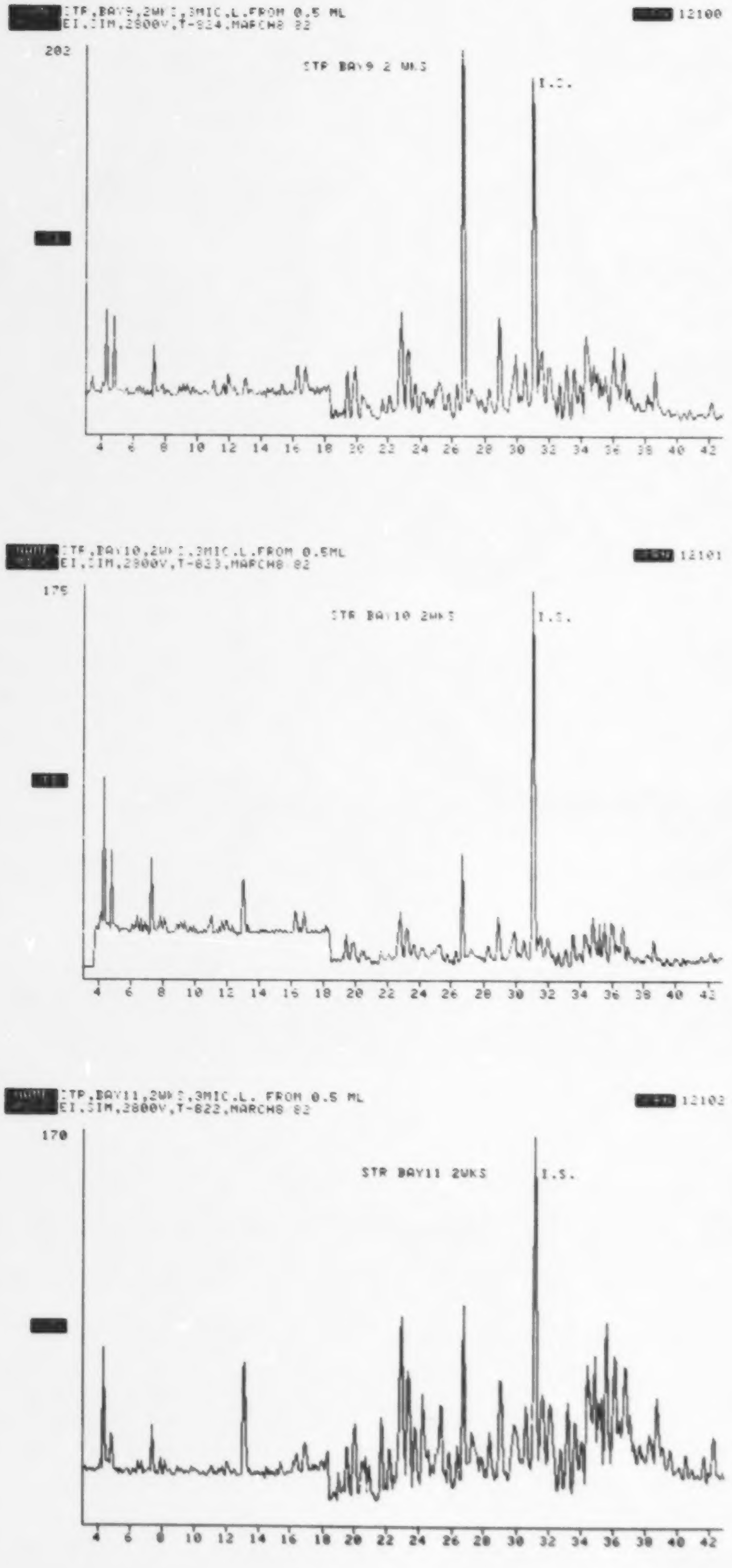


Figure 3.28 GC^2/MS total ion chromatograms of Strongylocentrotus droebachiensis tissues from urchin traps at 5 m depth in three bays

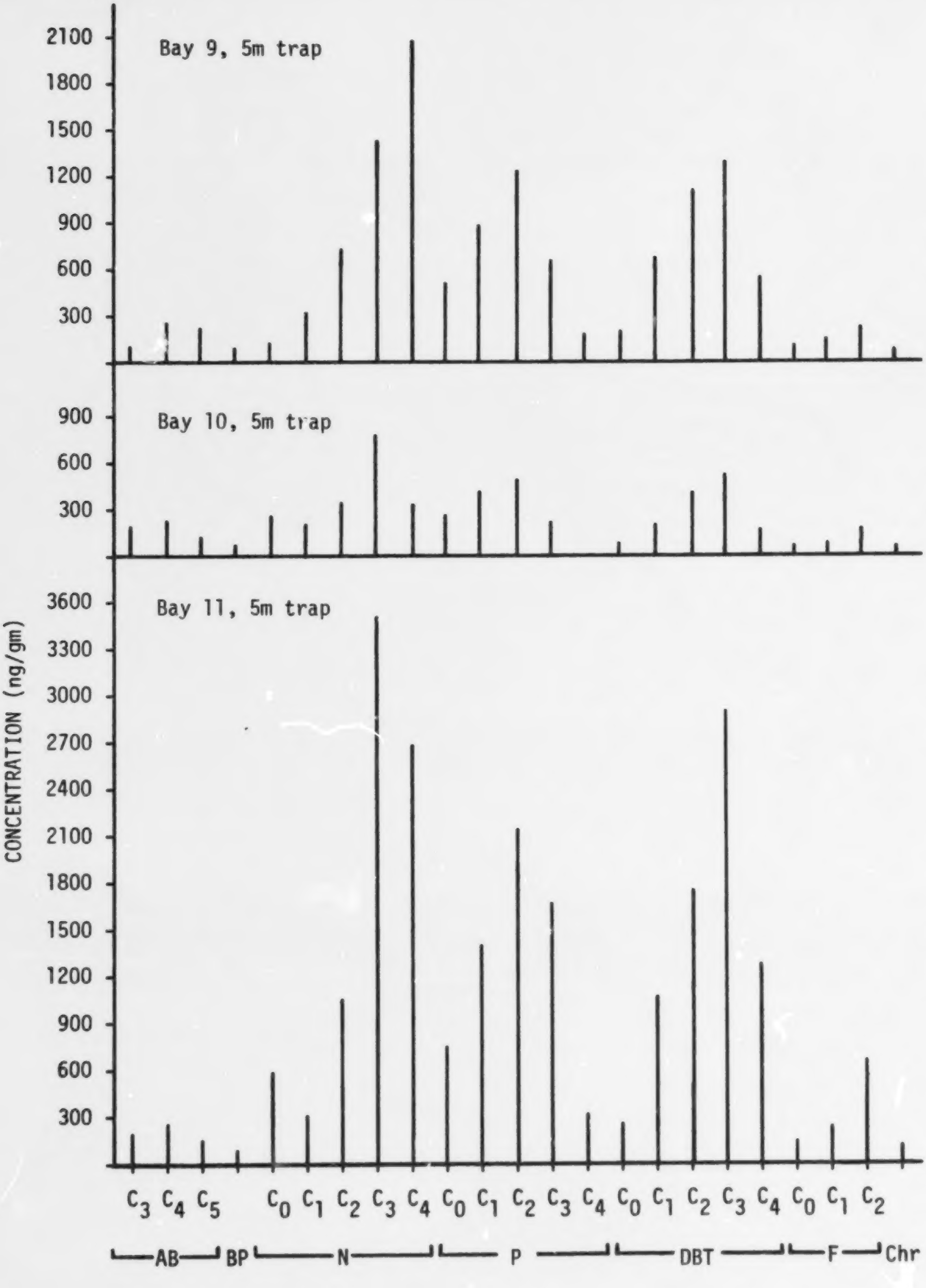


Figure 3.29 Aromatic profiles in Strongylocentrotus droebachiensis, 2nd post-spill sample

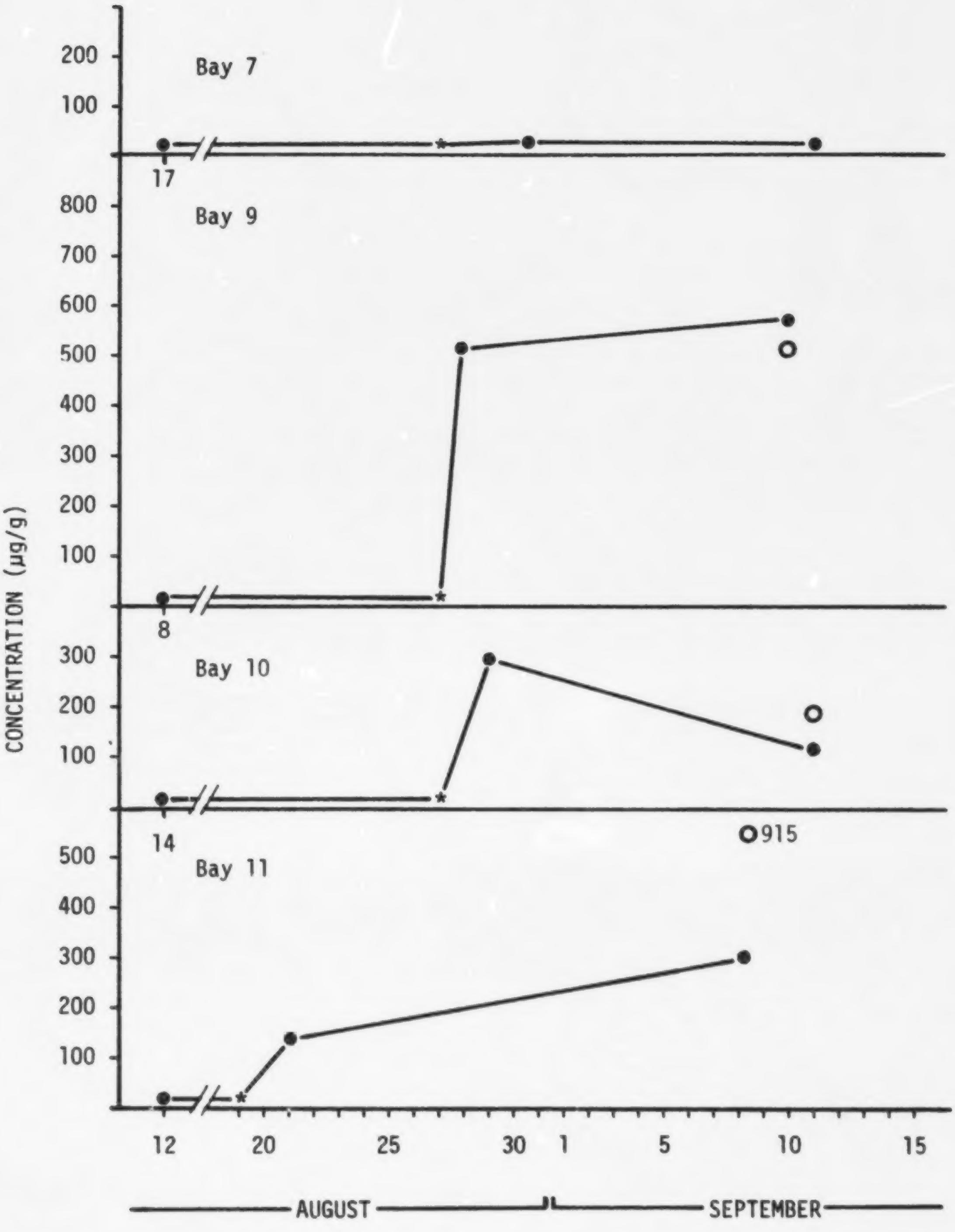


Figure 3.30 Trends in oil concentration in Strongylocentrotus droebachiensis, expressed as o-terphenyl equivalents (µg/g dry weight), (*, day of spill; O, 5m trap sample)

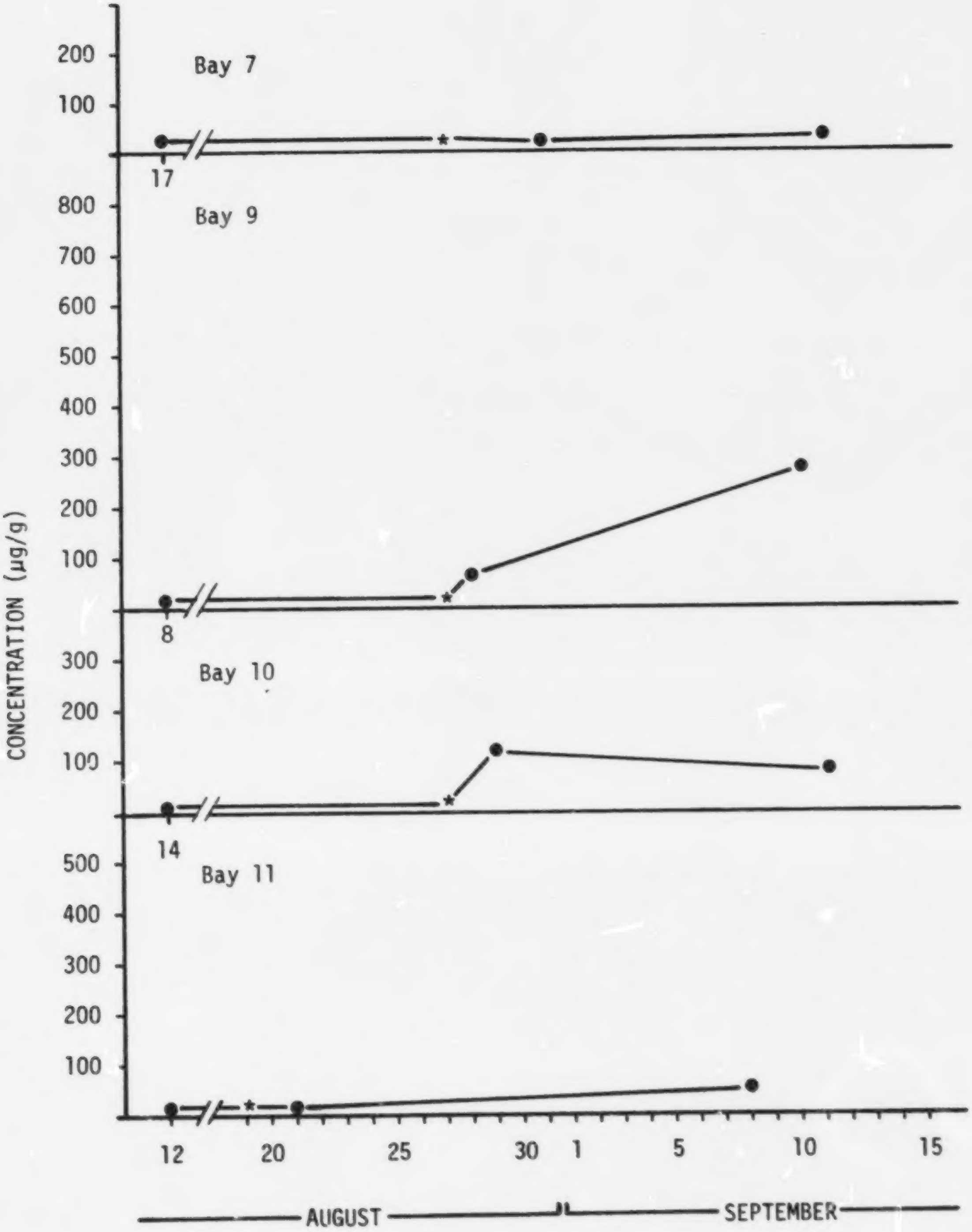


Figure 3.31 Trends in oil concentration in Pectinaria granulosa, expressed as oil equivalents (µg/g dry weight), by UV/F (*, day of spill)

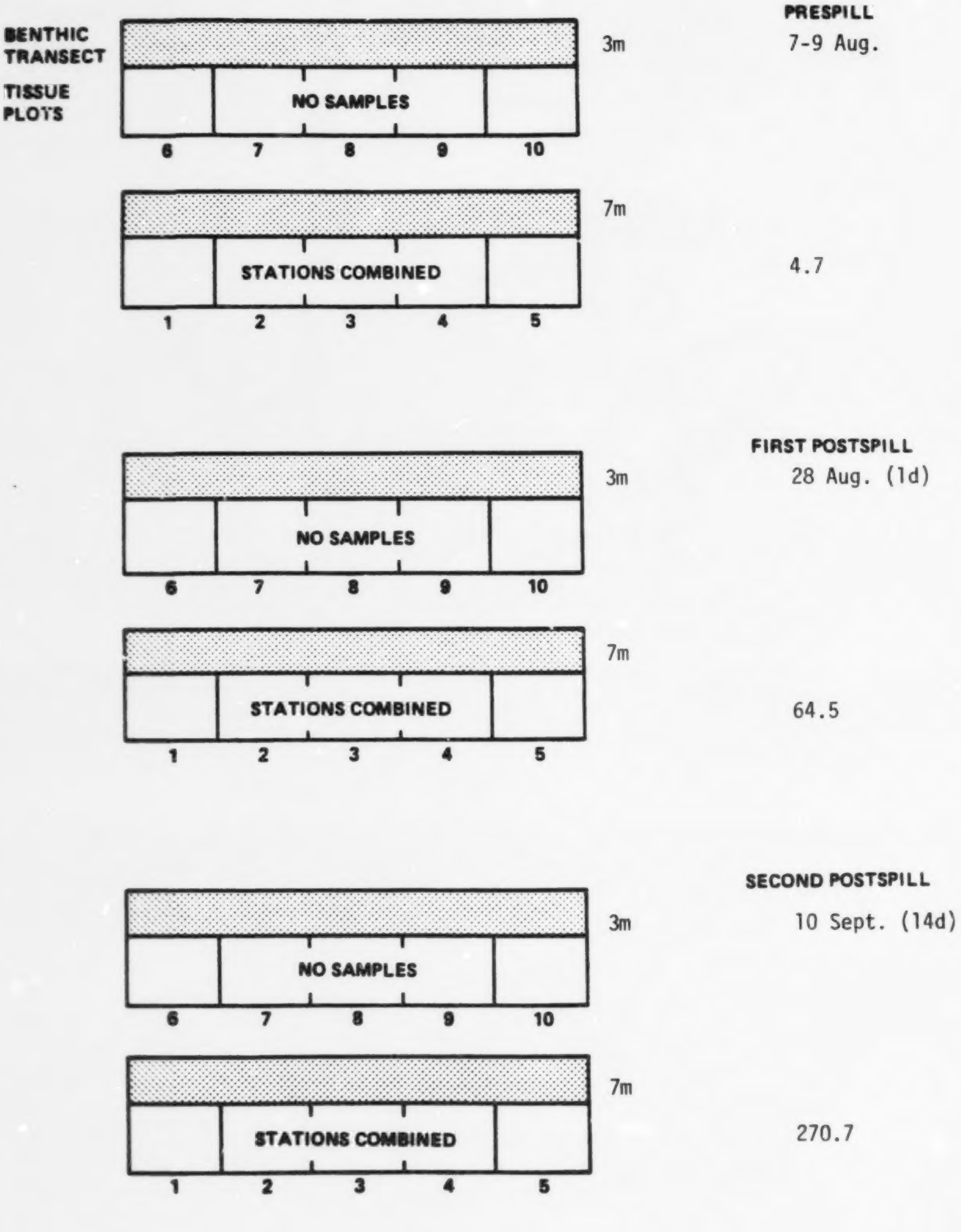

PRESPILL
7-9 Aug.
BENTHIC TRANSECT
TISSUE PLOTS
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
1
2
3
4
5
4.7
FIRST POSTSPILL
28 Aug. (1d)
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
1
2
3
4
5
64.5
SECOND POSTSPILL
10 Sept. (14d)
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
1
2
3
4
5
270.7

Figure 3.32 Concentrations of oil in Pectinaria granulosa from Bay 9, by UV/F (ug/g dry weight)

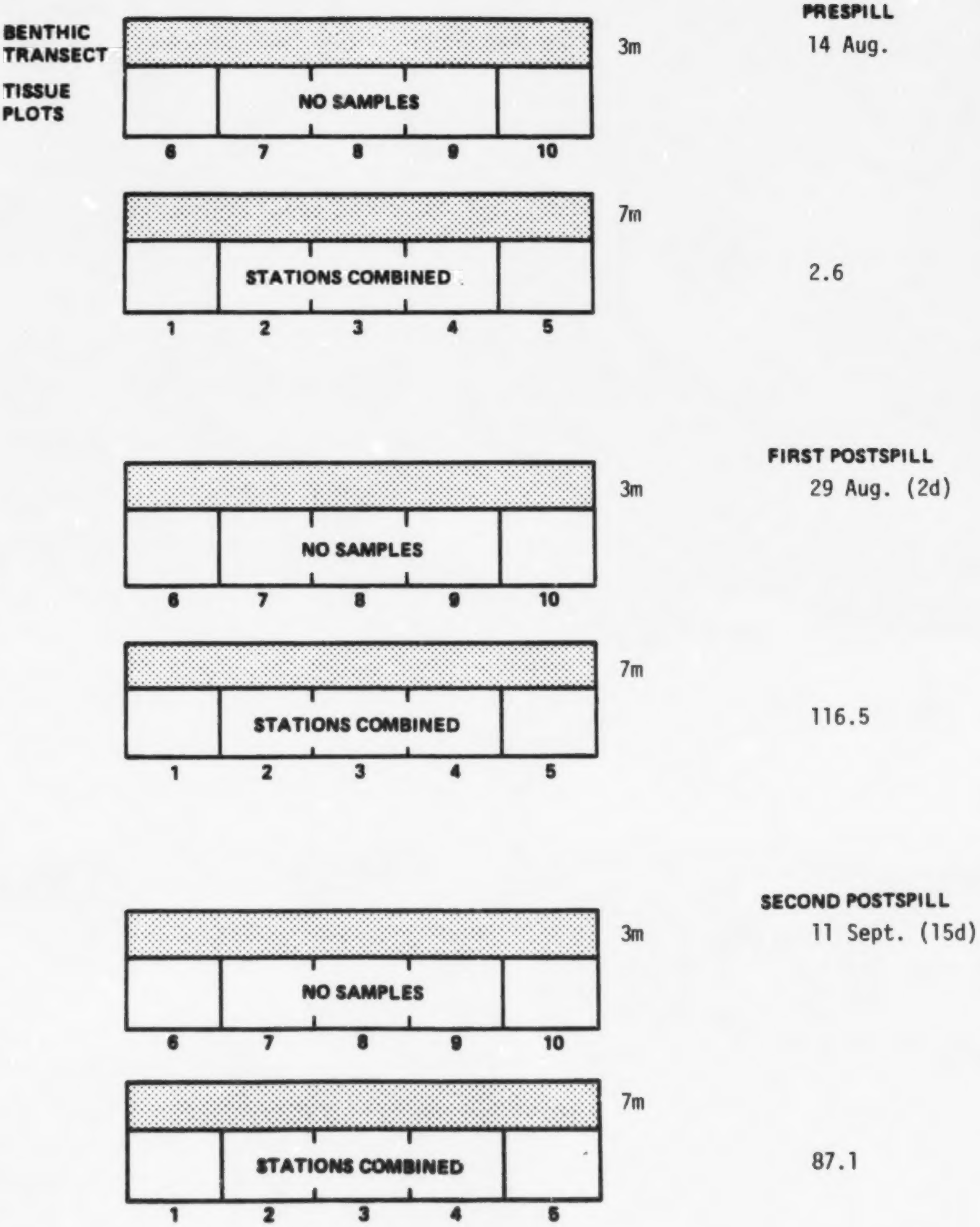

PRESPILL
14 Aug.
BENTHIC TRANSECT
TISSUE PLOTS
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
2.6
1
2
3
4
5
FIRST POSTSPILL
29 Aug. (2d)
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
116.5
1
2
3
4
5
SECOND POSTSPILL
11 Sept. (15d)
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
87.1
1
2
3
4
5

Figure 3.33 Concentrations of oil in Pectinaria granulosa from Bay 10, by UV/F (ug/g dry weight)

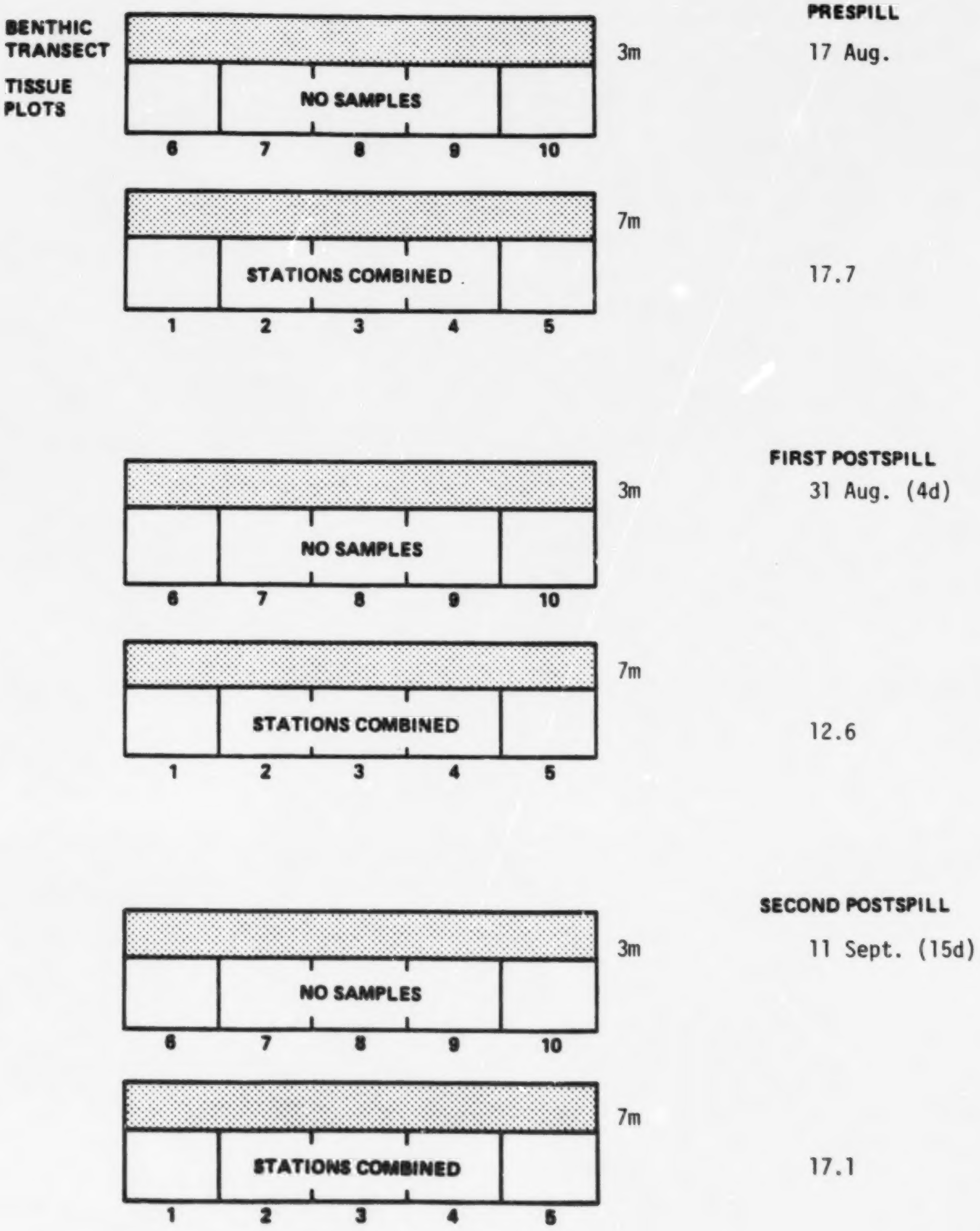

BENTHIC TRANSECT
TISSUE PLOTS
PRESPILL
17 Aug.
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
17.7
1
2
3
4
5
FIRST POSTSPILL
31 Aug. (4d)
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
12.6
1
2
3
4
5
SECOND POSTSPILL
11 Sept. (15d)
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
17.1
1
2
3
4
5

Figure 3.34 Concentrations of oil in Pectinaria granulosa from Bay 7, by UV/F (ug/g dry weight)

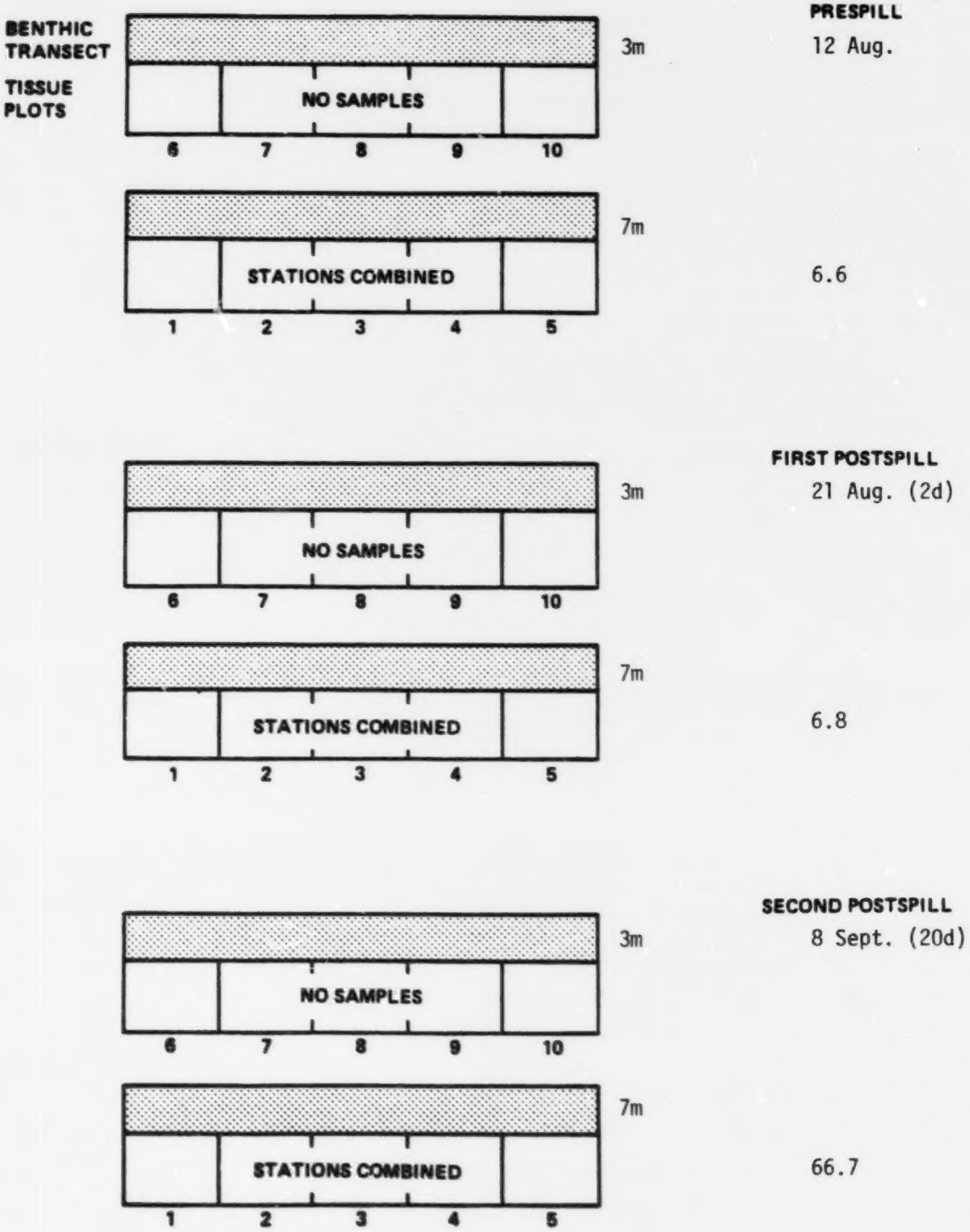

BENTHIC TRANSECT
TISSUE PLOTS
NO SAMPLES
6
7
8
9
10
3m
PRESPILL
12 Aug.
7m
STATIONS COMBINED
1
2
3
4
5
6.6
FIRST POSTSPILL
21 Aug. (2d)
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
1
2
3
4
5
6.8
SECOND POSTSPILL
8 Sept. (20d)
3m
NO SAMPLES
6
7
8
9
10
7m
STATIONS COMBINED
1
2
3
4
5
66.7

Figure 3.35 Concentrations of oil in Pectinaria granulosa from Bay 11, by UV/F (ug/g dry weight)

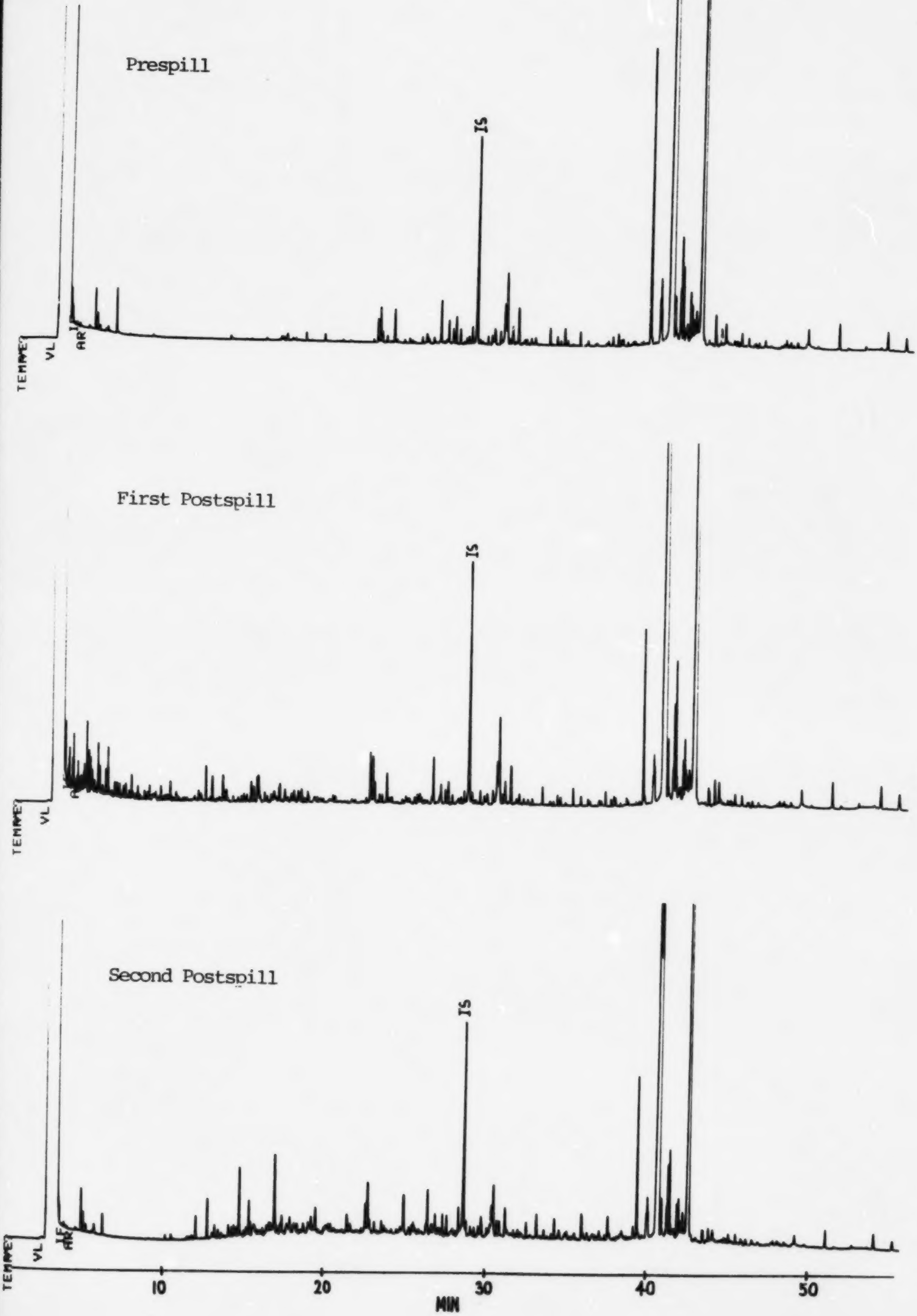


Figure 3.36 GC^2/FID chromatograms of *Pectinaria granulosa* tissues from Bay 9

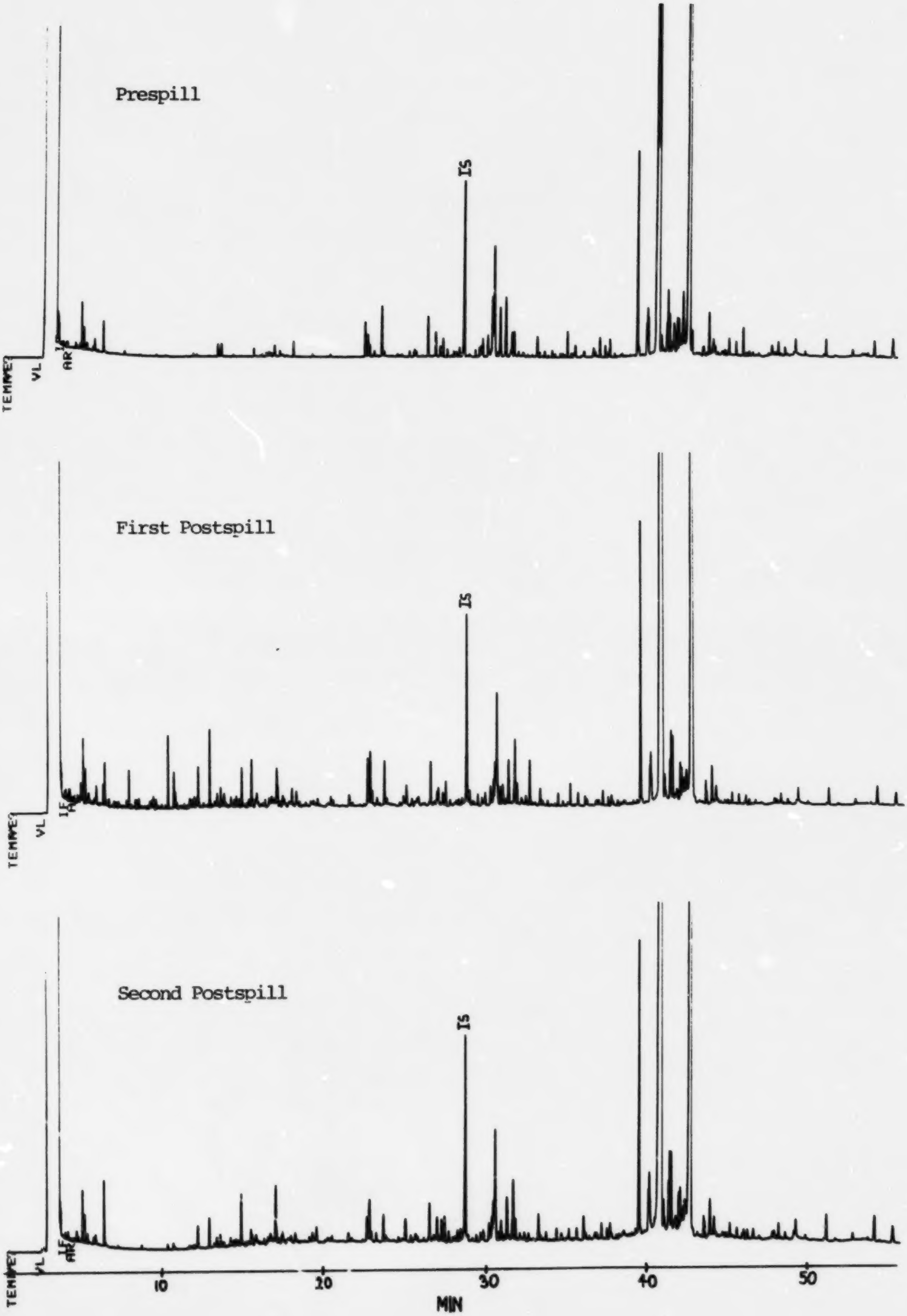


Figure 3.37 GC^2/FID chromatograms of *Pectinaria granulosa* tissues from Bay 10

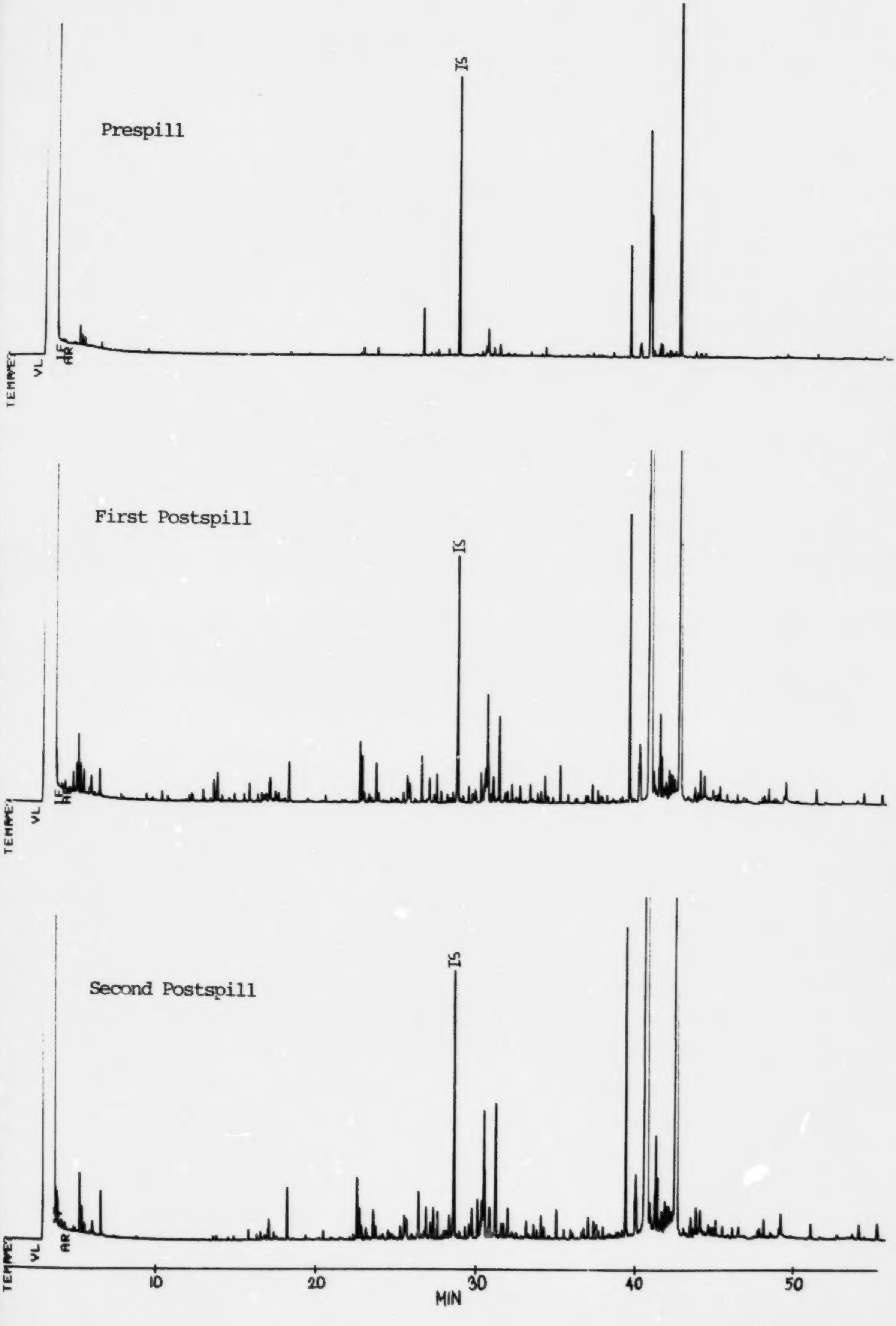


Figure 3.38 GC^2/FID chromatograms of Pectinaria granulosa tissues from Bay 7

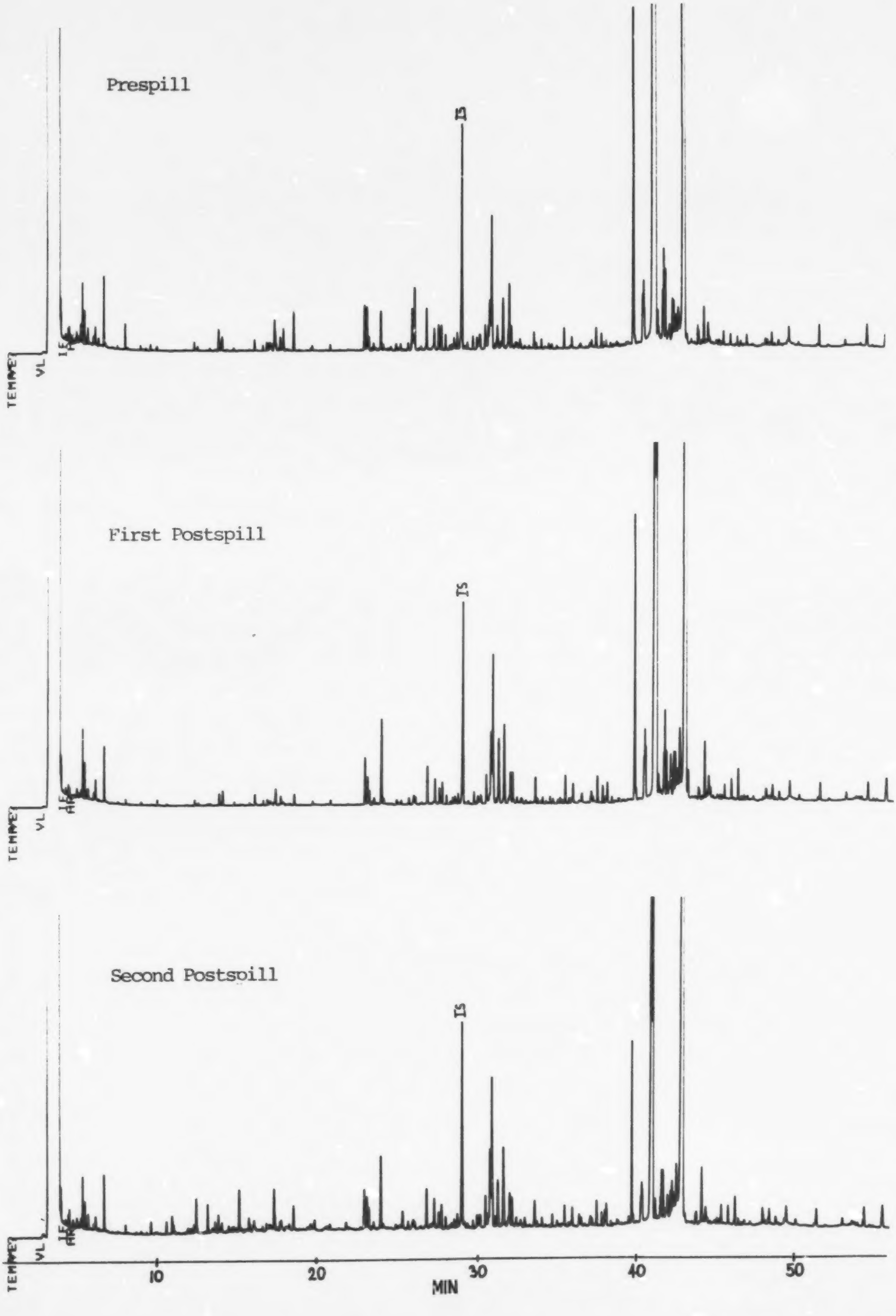


Figure 3.39 GC^2/FID chromatograms of Pectinaria granulosa tissues from Bay 11

Levels of pristane were low (1-2 ug/g), less than in urchins, and there was no discernible difference among bays (Table 3.3). Generally, phytane levels increased from trace levels found in pre- and first post-spill tissue samples. A general effect was a decrease in the calculated pristane/phytane ratios.

Although there were not quantitated, it is interesting to note the behaviour of the iso-alkanes in the C12-C15 region of the chromatograms. By the second time period post-spill, iso-alkanes with retention indices 1273, 1377 and 1462 (farnesane) were distinct peaks in the chromatograms of the polychaetes from Bays 9, 10 and 11. Farnesane in particular was more abundant relative to phytane in these samples than it was in the oil. Small peaks in the C12-C15 region were also found for urchins (Figures 3.10 to 3.15) but there was no evidence for preferential accumulation of lower molecular weight iso-alkanes in this species as is suggested for the polychaetes.

3.2.2.3 GC^2/SIM

Aromatic hydrocarbon profiles of polychaete tissues from Bay 9 showed a proportional emphasis on lower molecular weight aromatics in 1 day post-spill samples, shifting to an emphasis on larger molecular weight aromatics by 14 days post-spill (Figure 3.40). The relationships are presented in detail in SIM assessments, as shown in Figures 3.41 to 3.45. This set of chromatograms for SIM data is qualitatively representative of all polychaete and urchin samples analyzed for aromatic composition by mass spectrometry.

Bay 9 polychaete tissues showed high levels of C3- and C4- alkyl benzenes and C1-, C2- and C3- naphthalenes approaching 2000 ng/g in the first post-spill samples (Figure 3.46). Similarly high levels of C3- naphthalene and C2- phenathrene were found in second post-spill tissues. The poly-methylated phenanthrenes and dibenzthiophanes were markedly enhanced in the later sample.

Polychaete tissues from Bay 10 had an aromatic profile similar to that in Bay 9, but were of lower concentration (Figures 3.47 and 3.48). Bay 7 tissues showed no conclusive trend for alterations in aromatic composition or concentration, in agreement with the UV/F data (Figures 3.49 and 3.46).

TABLE 3.3 Pristane and Phytane levels (ug/g dry weight) and ratios in *Pectinaria granulosa*

Site	Time Re Spill	No. Indiv. in Pool	Pristane	Phytane	Pristane-Phytane Radio
Bay 9-7m	pre	50	1.24	<0.20	>6.2
	1st	48	1.54	<0.20	>7.7
	2nd	33	2.28	1.77	1.29
Bay 10-7m	pre	134	0.87	<0.20	>4.5
	1st	199	2.25	0.87	2.57
	2nd	37	1.61	0.85	1.89
Bay 7-7m	pre	9	1.36	<0.20	>6.8
	1st	90	1.80	<0.20	>9.0
	2nd	39	0.68	<0.20	>3.4
Bay 11-7m	pre	108	1.81	0.21	8.62
	1st	82	0.80	<0.20	>4.0
	2nd	66	1.36	0.74	1.84

---

[1] Percent water in soft tissue, 73.0 ± 4.9

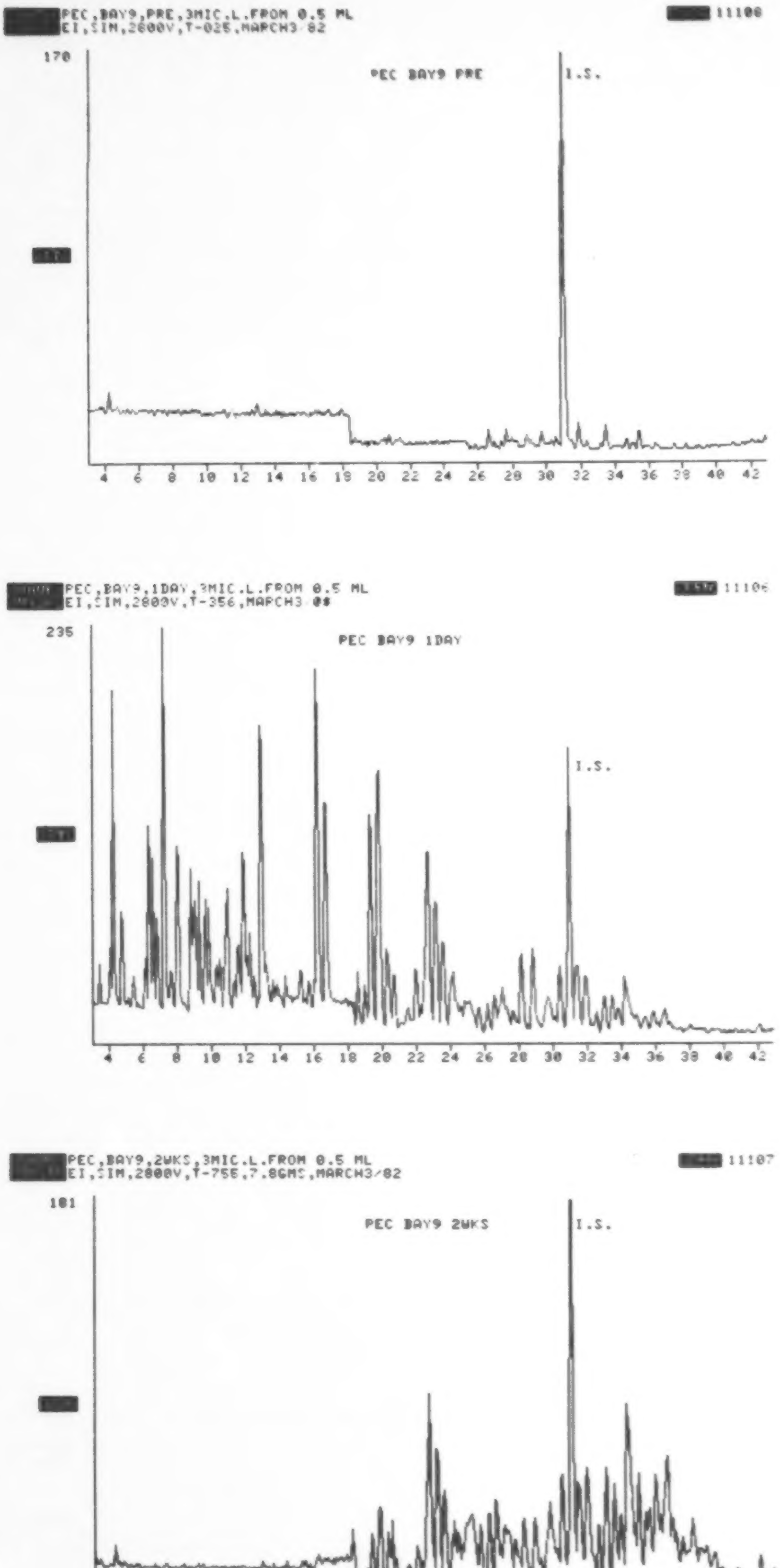


Figure 3.40 GC^2/MS total ion chromatograms of *Pectinaria granulosa* tissues from Bay 9

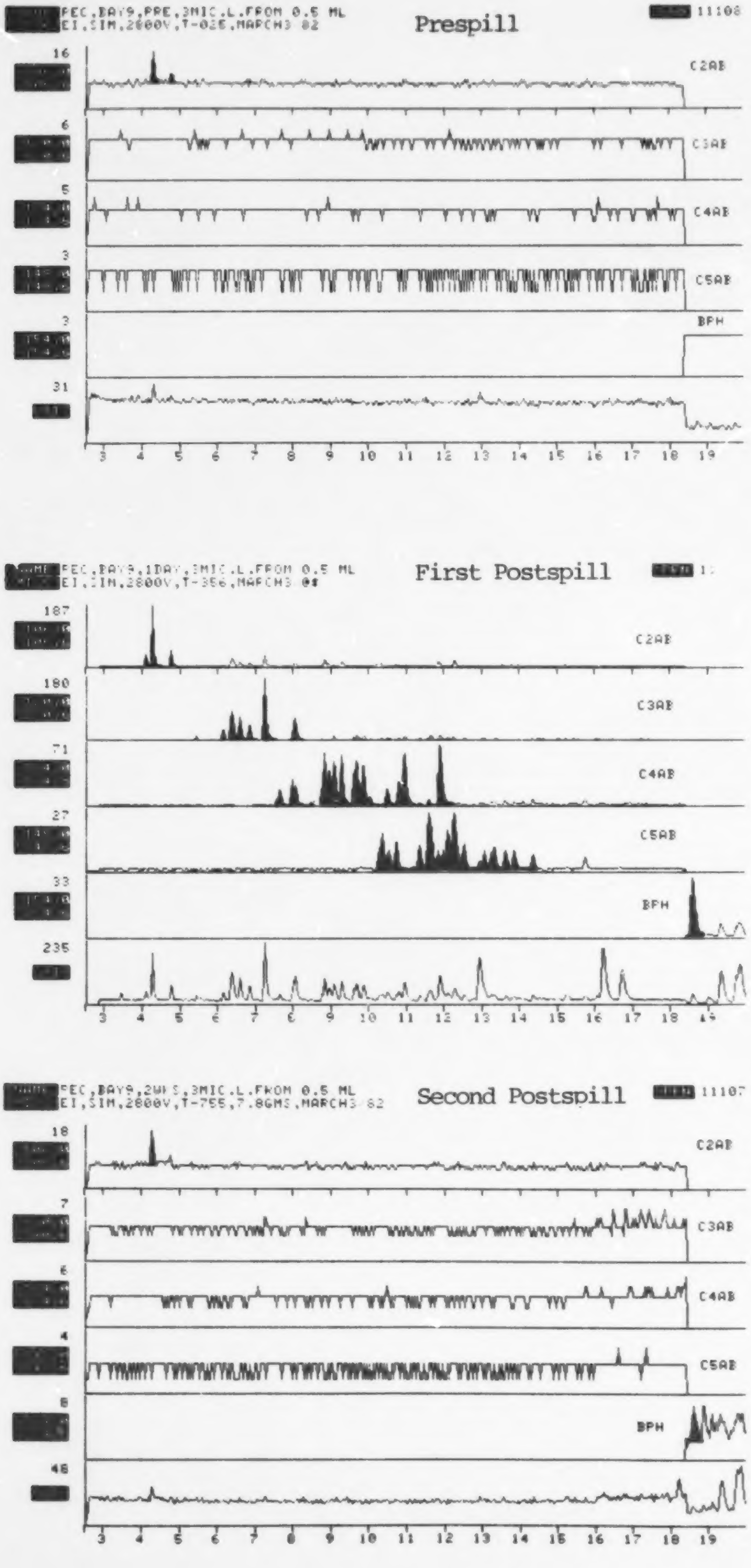
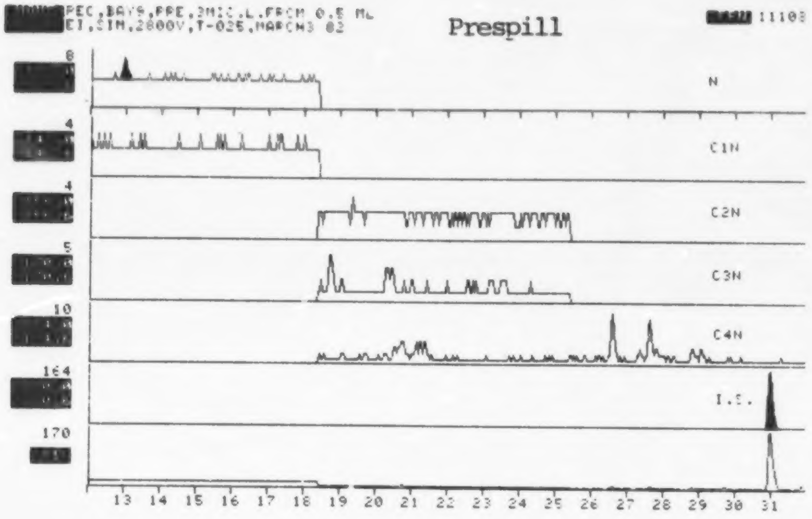
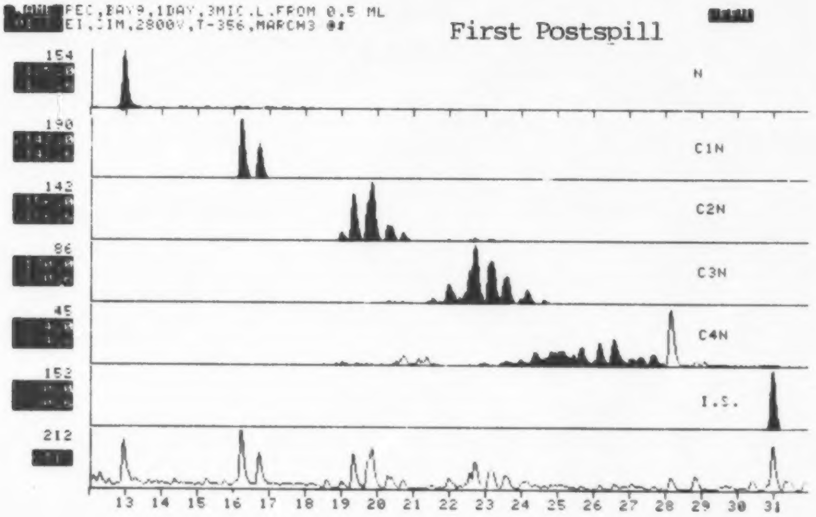


Figure 3.41 GC^2/SIM chromatograms showing benzenes and biphenyl of *Pectinaria granulosa* tissues from Bay 9

Prespill



First Postspill



Second Postspill

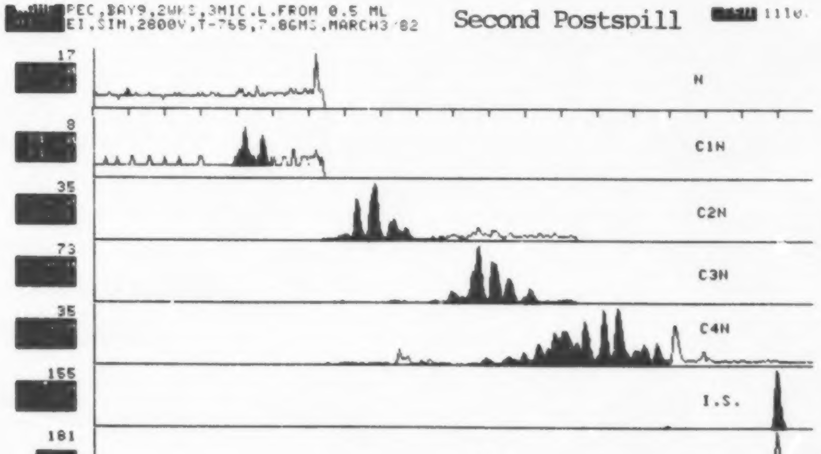


Figure 3.42 GC^2/SIM chromatograms showing naphthalenes of Pectinaria granulosa tissues from Bay 9

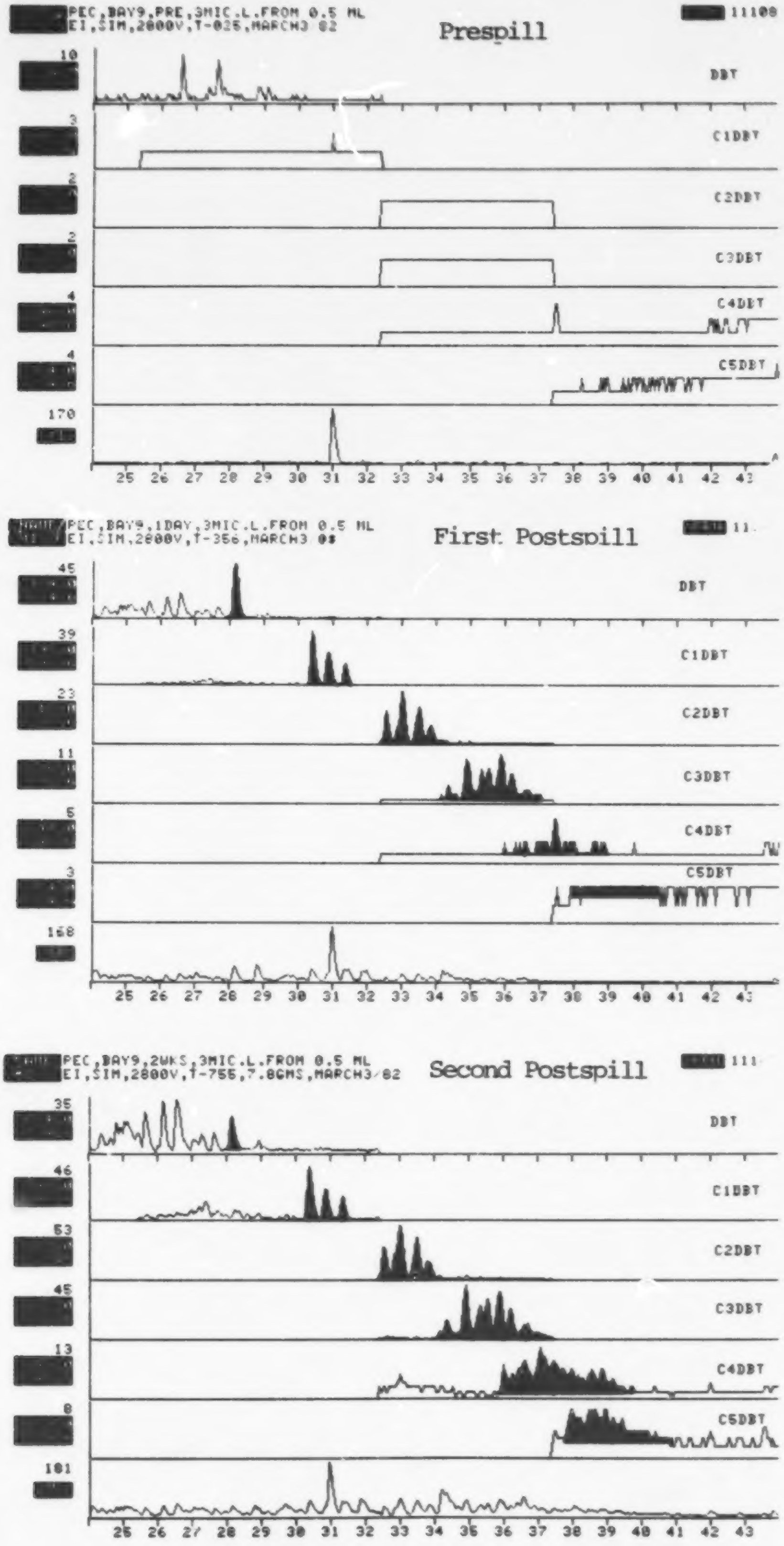
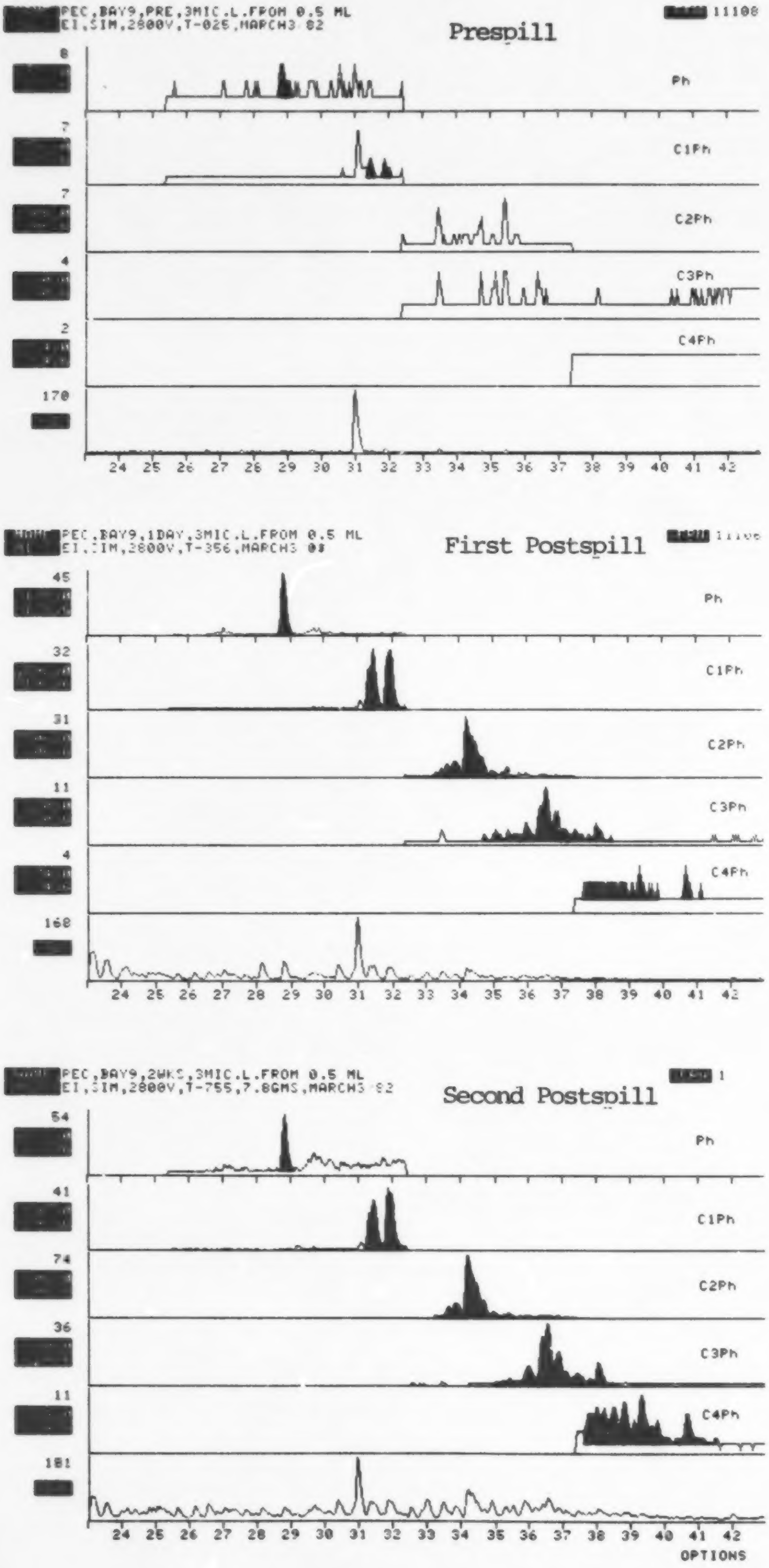


Figure 3.43 GC^2/SIM chromatograms showing dibenzthiophenes of Pectinaria granulosa tissues from Bay 9



Prespill

First Postspill

Second Postspill

Figure 3.44 GC^2/SIM chromatograms showing phenanthrenes of Pectinaria granulosa tissues from Bay 9

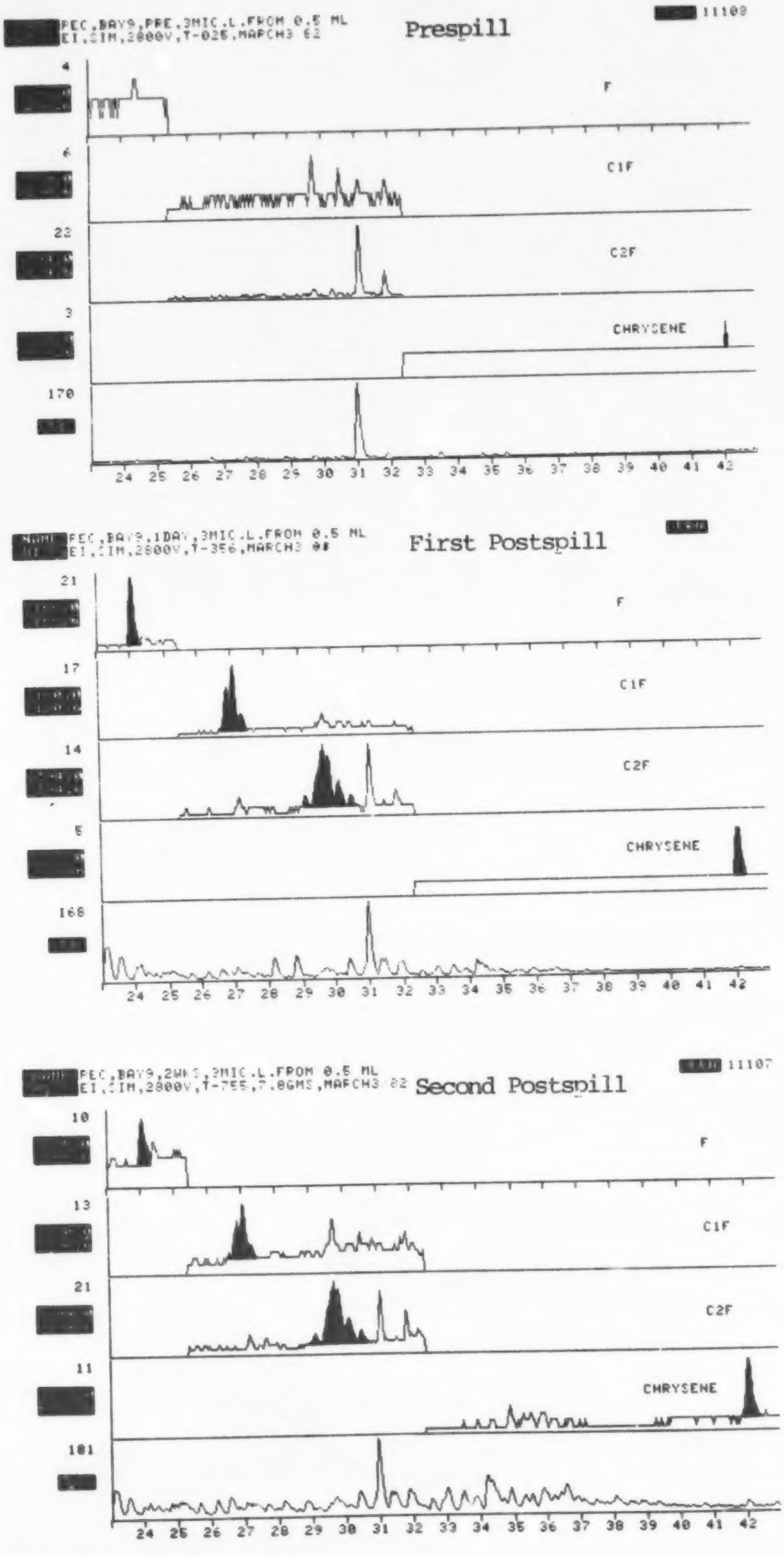


Figure 3.45 GC^2/SIM chromatographs showing fluorenes and chrysene of *Pectinaria granulosa* tissues from Bay 9

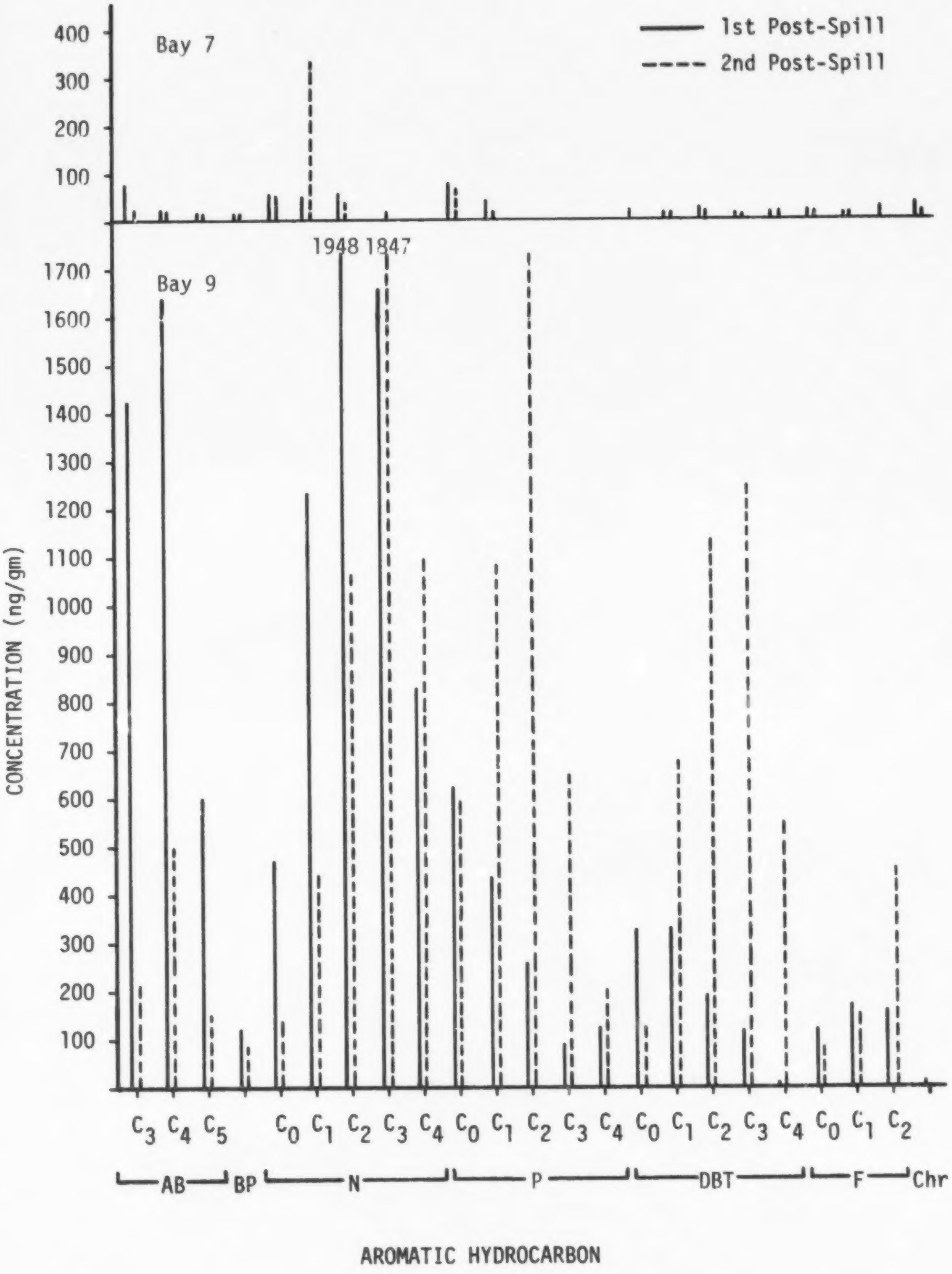


Figure 3.46 Aromatic profiles in Pectinaria granulosa

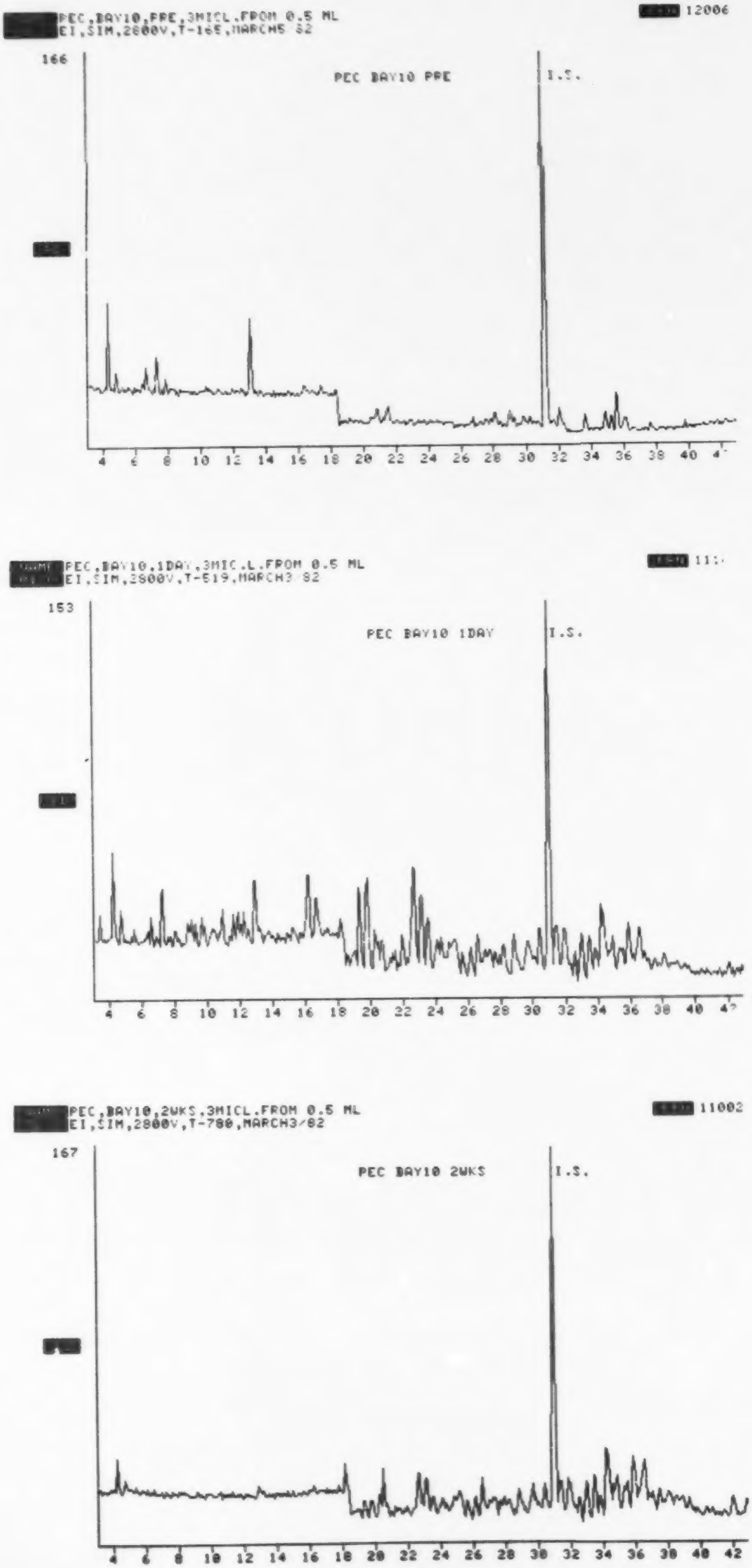


Figure 3.47 GC^2/MS total ion chromatograms of Pectinaria *granulosa* tissues from Bay 10

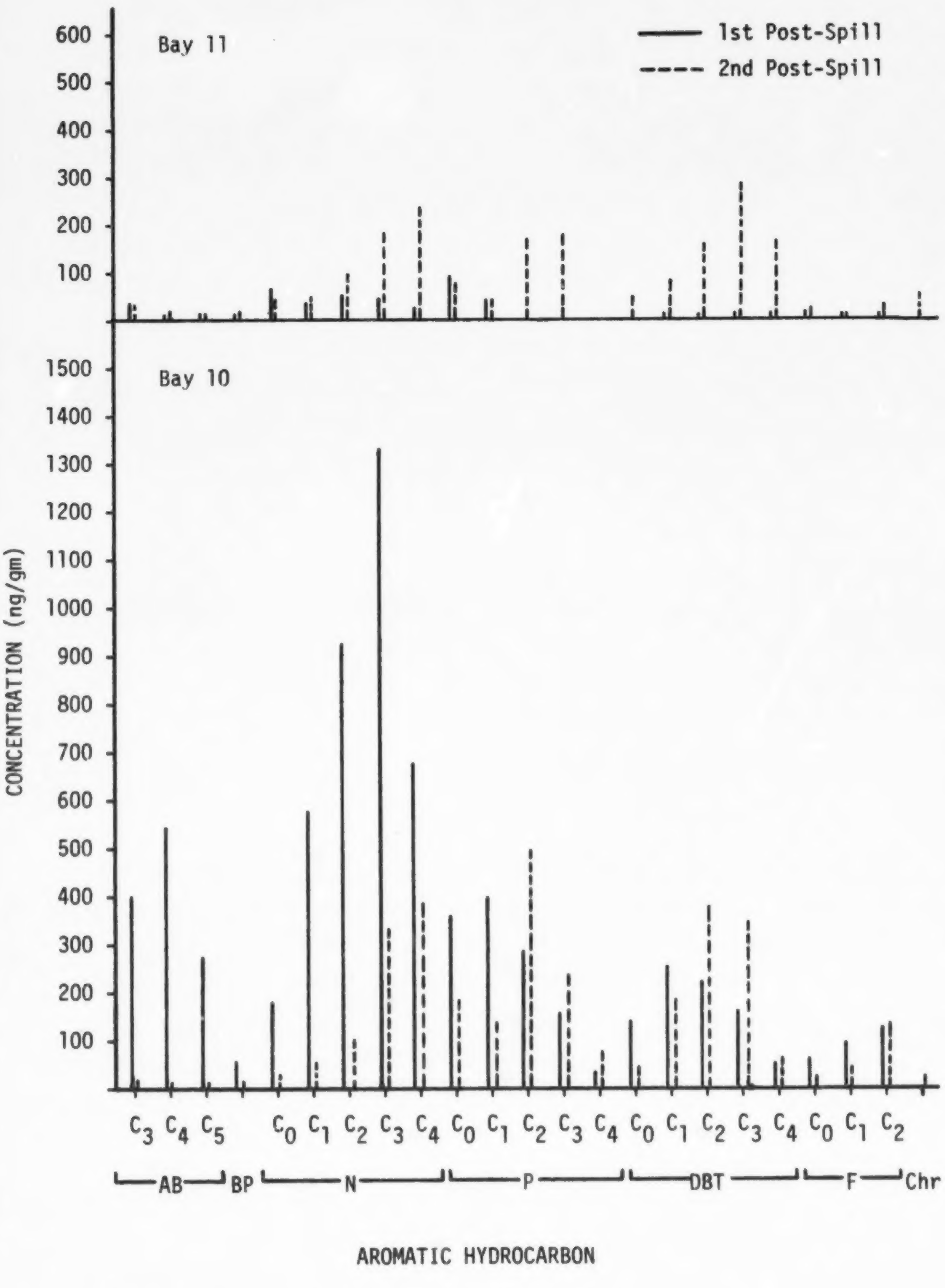


Figure 3.48 Aromatic profiles in Pectinaria granulosa

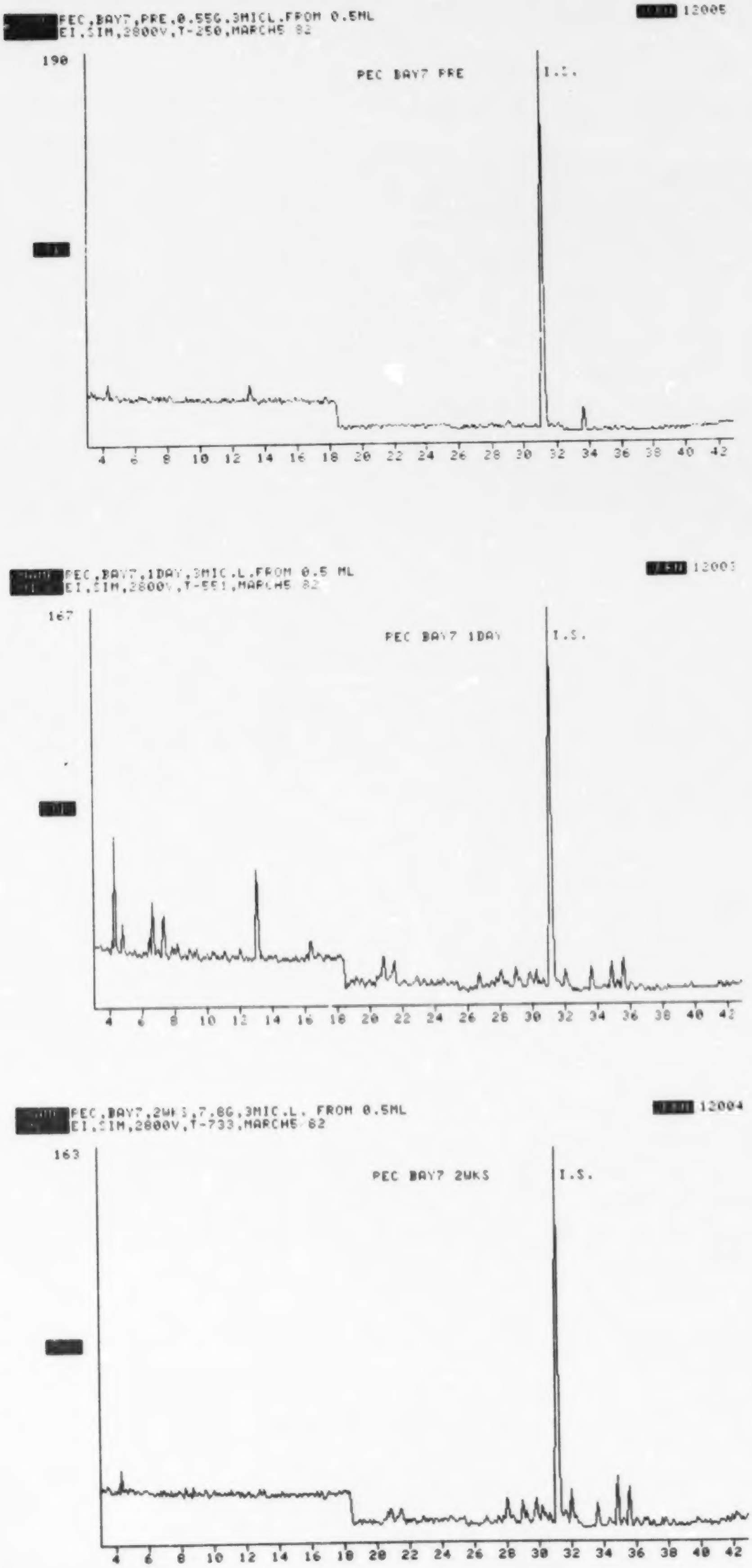


Figure 3.49 GC^2/MS total ion chromatograms in Pectinaria granulosa tissues from Bay 7

Analysis of tissues from Bay 11 polychaetes demonstrated a relative emphasis on polymethylated and polynuclear aromatic hydrocarbons in the second post-spill sample (Figures 3.50 and 3.48).

A comparative calculation of total oil concentration using aromatic data was carried out for polychaete samples as well. Total tissue oil levels were greater when expressed on this basis (Figure 3.51) than on the basis of fluorescence (Figure 3.31). This was especially true for Bay 9 and Bay 10 post-spill samples, in particular Bay 9 first post-spill.

3.3 Laboratory Intercalibration

The results are shown in Tables 3.4 and 3.5. In all cases there was close agreement between oil equivalent concentrations derived from n-alkane (FID) and PAH (SIM) analysis. Average concentrations from GC^2 analysis of the oil spiked urchin and *Mya* tissues, as well as Lagomedio oil, were 23.4, 40.7 and 48.7 ug/ml respectively. This indicates reasonably good agreement with the presumed concentrations of total hydrocarbons in the unknown samples, taking into consideration the amount of the oil spike and background hydrocarbon concentrations in the tissue extracts.

Oil concentrations determined by UV/F agree in orders of magnitude with GC^2-derived concentrations and with presumed background oil levels in the tissues prior to spiking. They are, however, less consistent in an absolute sense. Oil spill-derived urchin samples were under-estimated, similar to the situation described previously in Section 3.2.

The unknown *Mya* extract concentration was probably over-estimated by UV/F, although there was close accord in the oil-only sample concentration.

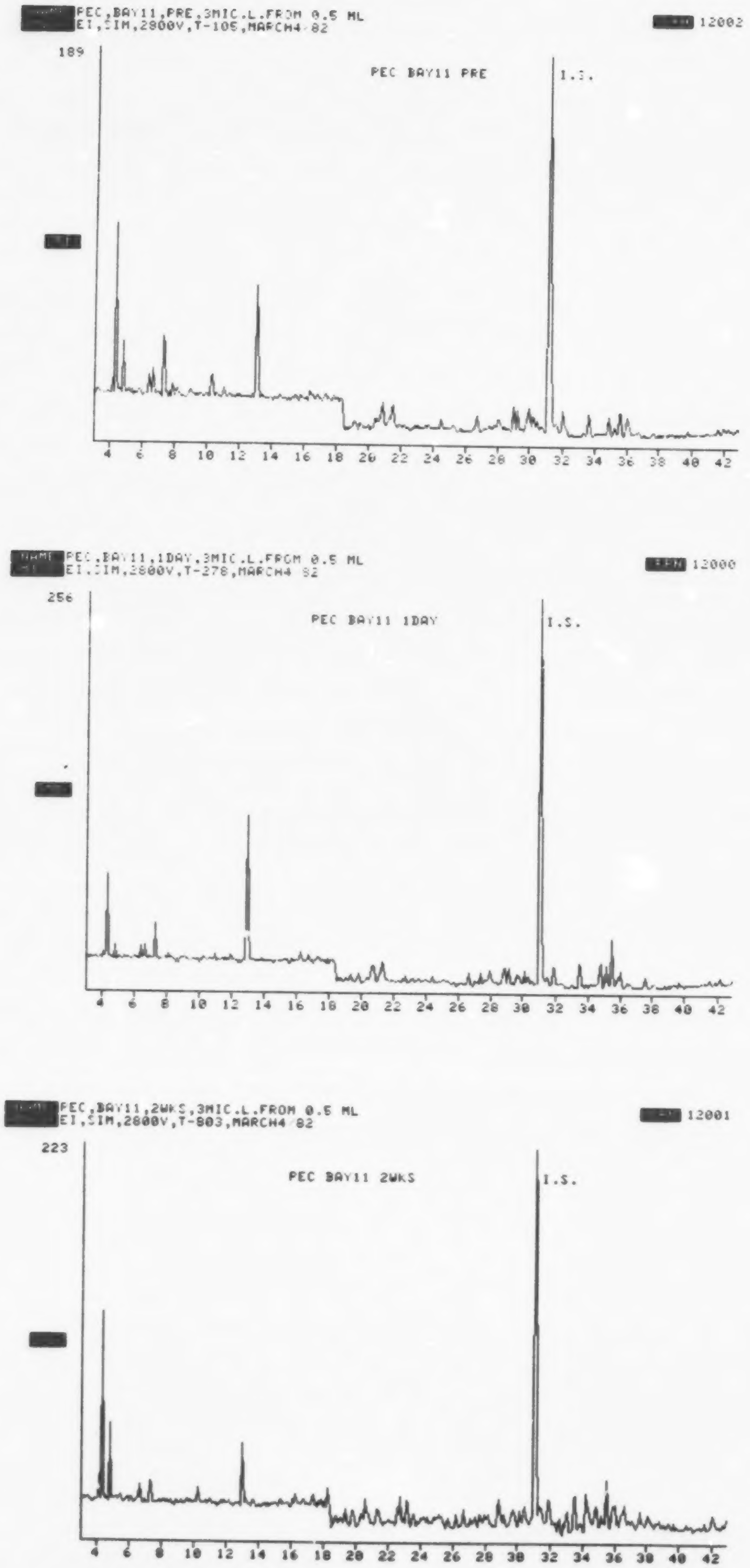


Figure 3.50 GC^2/SIM total ion chromatograms in Pectinaria granulosa tissues from Bay 11

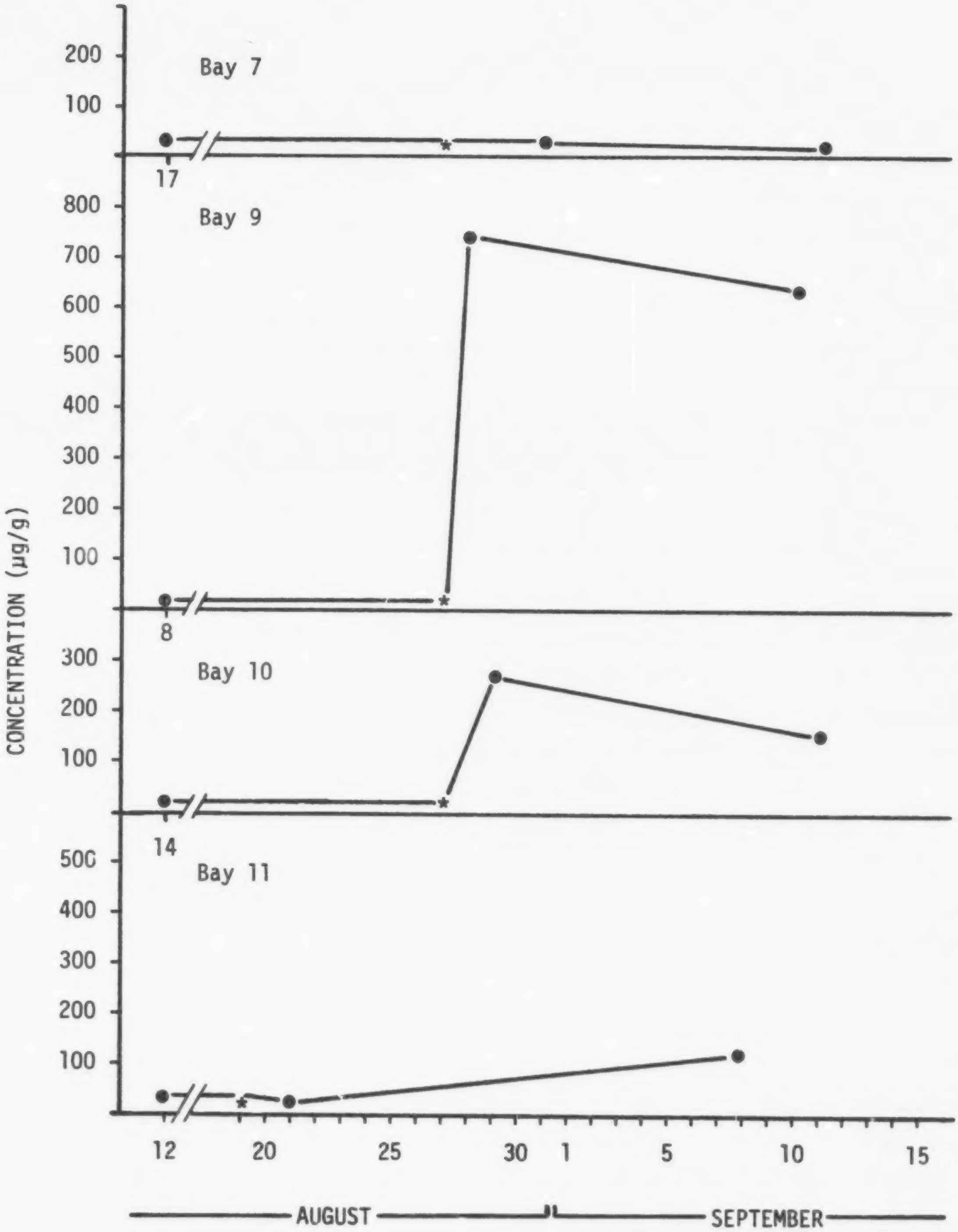


Figure 3.51 Trends in oil concentration in Pectinaria granulosa, expressed in o-terphenyl equivalents (μg/g dry weight) (*, day of spill)

Table 3.4 Results of CWS/DIAND analysis of BIOS intercalibration samples, oil equivalent concentrations.

Unknown[1] Sample	Amt.Oil Added (ug/ml)	Duplicate No.	Androstane Recovery(%)	Concentration of Oil im ug/ml[2] calculated from		
				Alkanes[3]	PAH[4]	UV/F analysis[5]
1	0	1			299	93.1
2	20.7	1	86.9	23.8	23.6 ± 2.5	38.8
		2	86.7	23.3	22.8 ± 2.8	39.0
3	29.2	1	98.4	44.8	38.8 ± 1.4	75.2
		2	107.3	42.7	36.6 ± 7.4	72.0
4	34.1	1	88.7	42.9	50.8 ± 3.9	31.7
		2	96.9	47.2	48.8 ± 2.8	33.7

[1] Sample 1 - urchin homogenate, Bay 10 first post-spill, 0.2 g dry weight/ml.
2 - urchin extract, pre-spill spiked, 0.60 g. net weight/ml
3 - *Mya* extract, approx. 0.35 g net weight/ml
4 - Lagomedio oil in solvent

[2] Except for sample 1, where concentration is ug/g dry weight

[3] Based on sum of peak heights of C18 - C22 n-alkanes compared to oil standard by FID

[4] Based on areas of individual PAH (C1-C2-naphthalenes, phenanthrenes and dibenzthiophenes) by SIM, mean ±5.0

[5] Excitation 300 nm, emission 350 nm

SECTION 4 - DISCUSSION

4.1 Dispersed Oil Exposures

Both S. droebachiensis and P. granulosa evidenced a rapid uptake of Lagomedio oil into soft tissues following release of the dispersion. Animals taken from Bay 9, the site of the dispersed oil release, had higher concentrations than those from Bay 10 which received the dispersed oil by current diffusion within about one-half day. Green et al. (1982) report that Bay 9 oil levels were of the order of 300 to 400 ppm-hours, measured over 36-hours, while Bay 10 levels were about 30 ppm-hrs.

Bay 7 received appreciably less oil over the 36 hour time period, resulting in an exposure level of 0.5 ppm-hrs. This lower level is reflected in the low tissue hydrocarbon levels found in urchins and particularly in polychaetes from Bay 7.

A distinguishing characteristic of the tissue hydrocarbon load accumulated initially from dispersed oil is the relatively high concentration of low molecular weight hydrocarbons. The shift to larger molecular weight and polymethylated aromatics about two weeks after the spill suggests an enhanced loss of the former component, although total oil quantities showed only small or no decrease in the second post-spill samples. A continued increase in tissue levels is probable in Bay 9 urchins and polychaetes. This suggests continued accumulation from the substrate or from food supplies since water levels of hydrocarbons become negligible within two days time after the spill.

It is interesting to note that pristane levels in urchins from Bays 7 and 9 were twice as high as those from Bays 10 and 11: 22±3 and 25±3 as compared to 13±1 and 10±1 ppm, respectively, from the means of the pre-, first and second post-spill determinations. This points out that there is probably a difference in feeding ecology for this species in these two areas. There was no discernable difference between Bays in the case of polychaetes. Generally, phytane levels increased in both species from trace levels found in pre- and first post-spill, to ppm levels in the second post-spill sample.

At the second time post-spill, the phytane/n-C18 alkane ratio was higher in animal tissues than it had been previously, and as compared to that in the Lagomedio oil. This was especially true in the Bay 9 and 10 urchin trap samples. It is probable that biodegradation occurred over the approximately two week post-oiling period. The origin of enhanced tissue isoalkanes may have been either from endogenous biodegradative processes, in particular gut flora, or from ingestion or absorption of biodegraded oil from the substrate or food material. It had been reported by divers carrying out observations after the oiling event that by the second sampling period, urchins were feeding on incapacitated bivalves. The polychaetes, however, are considered to be surface deposit feeders.

A general conclusion which can be drawn from the dispersed oil spill test is that both S. droebachiensis and P. granulosa have a capacity to accumulate hydrocarbons to a high degree from either the water column or the sediment substrate when the oil is in particulate dispersed form. Later accumulation when the spilled oil has mainly disappeared from the water column may continue from the substrate and by ingestion of food material.

4.2 Oil Slick Exposures

In contrast to the generally similar hydrocarbon uptake picture shown by urchins and polychaetes when exposed to dispersed oil, the two species were found to differ in their response to the surface oil spill of Bay 11. Urchins showed a much greater degree of tissue accumulation under these conditions than did P. granulosa. It may be suggested that this difference is due to both the physiology and habitat of the species. The urchin would see an early continuous exposure to hydrocarbons dissolved in the water column because of its extensive water-vascular system. High levels in the second post-spill urchin sample may be attributed to either continuing absorption from very low background levels coupled with extensive retention of accumulated hydrocarbons, or as is more probable, a continued input from food sources. The sediment itself was of low contamination (Boehm et al. 1982) and had probably little effect. Low oil levels in the sediment of Bay 11, especially as compared to Bays 9 and 10, explain the low tissue oil levels found in P. granulosa since this species is closely tied to the substrate for both habitat and food supply.

It is most interesting that urchins from Bay 11 had the highest tissue hydrocarbon levels of all four bays, although the concentrations in this bay were several orders of magnitude lower than that found at 5 or 7 m in Bay 9 (Green et al. 1982). The oil levels are not likely due to extraneous contamination during sampling since the collection techniques guarded against such an occurrence. Further, the absence of low boiling hydrocarbons in the first post-spill sample set indicates that it was an uptake phenomenon which was described by the hydrocarbon data, not incidental contamination.

At present, there is no ready explanation for the marked differences in tissue oil levels noted between the five stations of Bay 11. There is some correlation with the sediment data which identified high hydrocarbon levels at the same sites (Boehm et al. 1982). Presumably some physical factor such as current eddies or tide channels may have played a role in causing the station heterogeneity along the 7m depth stratum.

One may conclude that a surface oil spill which was not treated with chemical dispersants (although in this case much of the spilled oil was mechanically removed) leads to a high degree of accumulation of larger molecular weight aliphatics and aromatics in S. droebachiensis. This occurred to a greater degree than when the oil was chemically dispersed, even though in this case water and sediment oil levels were much higher. Given the general consistency in tissue hydrocarbons in urchins from a 5m trap as compared to freely mobile urchins from the same bay, one can conclude that there has been little movement of urchins among bays or into a given test bay from uncontaminated areas.

In contrast to the urchin, the polychaete P. granulosa exhibited less tissue uptake in the surface spill condition as compared to dispersed oil exposures. This finding is in agreement with low water and sediment hydrocarbon burdens.

4.3 Laboratory Intercalibration

Results from the CWS FID and SIM analyses agreed well one with the other and were also similar to the presumed hydrocarbon levels in the unknown samples. That the total oil concentrations calculated on the basis of alkanes and PAH were greater than the amount of oil added may have been due to background hydrocarbons in the tissues prior to spiking, or be a reflection of the apparent 50% enhancement in concentration of the oil-only check sample. The UV/F assessments agreed well with presumed real concentrations in the case of the oil sample, but gave enhanced values for the spiked urchins and Mya samples. This is in accord with the recognized limitations of the UV/F assay, a method which may be more useful for screening purposes, yielding information on relative concentrations simply and rapidly.

The same check samples were assessed by ERCO (Boehm et al. 1982). Based on the concentration data presented from fluorescence analysis, it is apparent that values derived by this method by the laboratory underestimate hydrocarbon concentrations, although the oil-only unknown corresponds closely to true values, and is similar to the CWS/DIAND analysis. Both the spiked urchin and spiked Mya samples by UV/F were found to be a factor of three lower than the data generated the CWS/DIAND UV/F method. Analysis by ERCO for the unspiked urchin sample gave a result two times greater than that of the CWS/DIAND assay. These inconsistent differences in UV/F serve to point out the difficulties in comparing data between laboratories, even though methods were standardized as much as possible in this project. While an absolute transfer of data is thus not advisable, this comparison of UV/F data does point out the utility of the fluorescence method for screening.

SECTION FIVE - REFERENCES

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Boehm, P.D., D.L. Fiest, and P. Hirtzer. 1982. Baffin Island Oil Spill Project - Chemistry Component - Report on the 1981 Oil Spill Experiment. Vol. 2: Summary of Analytical Biogeochemistry. Final Report to the Environmental Protection Service, Department of the Environment Canada, May 1982.

Cross, W.E., and D.H. Thomson. 1982. Effect of Oil and Dispersed Oil on Nearshore Macrobenthos at Cape Hatt, Northern Baffin Island. II. Results of 1980 and 1981 Pre- and Post-Spill Studies. BIOS Project Report for the Environmental Protection Service, Departmnent of the Environment Canada, April 1982.

Green, D.R., B.H. Humphrey, and B. Fowler. 1982. Report on the 1981 Oil Spill Experiments. Vol. 1: Summary of Field Work. BIOS Project Report for the Environmental Protection Service, Department of the Environment Canada, March 1982.

		PERCENT RECOVERY [1]																		
SAMPLE TYPE	Spike ug Oil/ g net wt.	IS	C4 AB	BP	N	C1 N	C2 N	C3 N	C4 N	DBT	C1 DBT	C2 DBT	C3 DBT	Ph	C1 Ph	C2 Ph	C3 Ph	F	C1 F	C2 F
S. droeb.	100	106	-	75	15	11	49	77	91	97	106	119	144	123	129	120	122	98	125	209
" "	100	99	56	106	86	90	95	94	-	84	91	94	99	107	110	98	91	86	93	144
" "	50	98	104	141	129	89	92	97	51	50	109	91	26	106	102	81	-	130	-	111
" "	50	105	97	124	89	95	89	86	61	54	28	-	-	62	63	79	-	-	-	-
P. gran.	100	96	116	110	206	83	89	83	112	102	106	111	135	114	142	71	136	-	-	-
Blank	100	104	70	80	92	72	71	72	102	87	87	89	119	106	92	110	80	103	97	148
Blank	50	96	42	85	75	67	71	72	76	84	81	87	-	111	94	102	136	-	-	-

[1] IS = internal standard (o-terphenyl), AB = Alkyl benzenes, N = naphthalene, DBT = Dibenzthiophene
Ph = phenanthrene, F = fluorene

STR, Day 10 1st post spill Duplicate No.	Oil Equivalents, (UV fluoresc., ug/g dry weight	ng/g dry weight[1]																							
		C3 AB	C4	C5	BP	N	C1 N	C2 N	C3 N	C4 N	DBT	C1 DBT	C2 DBT	C3 DBT	C4 DBT	Ph	C1 Ph	C2 Ph	C3 Ph	C4 Ph	F	C1 F	C2 F	Chr	Total Aroma-tic	Total PAH
P01	86.6	400	463	233	45	195	557	883	1288	757	125	270	216	137	NA	355	392	280	148	20	51	95	119	40	7069	5973
P02	92.7	263	364	230	60	153	522	919	1325	641	139	266	247	181	NA	385	417	310	150	29	63	111	85	NA	6860	6003
P03	95.8	607	766	347	68	215	698	1007	1503	675	134	245	210	162	34	341	380	263	156	20	63	77	153	8	8132	6412
P04	94.2	312	553	263	53	122	511	863	1192	618	134	238	235	162	43	324	392	261	148	NA	54	76	83	NA	6637	5509
Mean	92.3	395	537	268	56	171	572	918	1327	673	133	255	227	161	39	351	395	278	151	23	58	90	108	-	7175	5974
SD (±)	4.0	152	171	55	10	42	86	64	130	61	6	16	17	18	7	26	16	23	4	5	6	17	32	-	662	369
CV (%)	4	38	32	20	17	24	15	7	10	9	4	6	7	11	11	7	4	8	2	23	11	19	30	-	9	6

[1] AB = alkyl benzene
BP = biphenyl
N = naphthalene

DBT = dibenzthiophene
Ph = phenanthrene
F = fluorene
Chr = chrysene

In Situ STUDIES OF EFFECTS OF OIL AND DISPERSED OIL ON PRIMARY PRODUCTIVITY OF ICE ALGAE AND ON UNDER-ICE AMPHIPOD COMMUNITIES

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For

Supply and Services Canada
Ottawa, Ontario

July 1982

EXECUTIVE SUMMARY

Effects of *in situ* applications of oil and dispersed oil on the abundance and productivity of under-ice algae and on behaviour, mortality and distribution of under-ice amphipods were studied at Cape Hatt, northern Baffin Island. Immediate effects of oil on ice algae and amphipods were studied by injecting oil into small chambers on the under-ice surface. In addition, distribution and life history data were collected for amphipods occupying under-ice, intertidal and shallow sublittoral habitats. The latter component of the study utilizes the large-scale experimental spills carried out at the BIOS (Baffin Island Oil Spill) site in August 1981; we obtained pre-spill (May and August 1981) and post-spill (September 1981) data.

Field studies were carried out during 16-31 May, 10-19 August and 7-8 September 1981 from the BIOS project base camp located at Cape Hatt, Baffin Island. All under-ice and sublittoral sampling and experimental work was carried out by SCUBA divers working through holes in the ice (May) or from small inflatable boats (August and September). Studies on amphipod distribution and population structure were conducted in three small bays at a depth of 3-5 m, or on the ice undersurface at the same location; intertidal sampling was carried out on the beach between two markers, 150 m apart, which demarcated the BIOS study bays. Experimental studies on amphipods and ice algae were conducted on the under-ice surface in another two bays over water depths of 8 and 12 m, respectively.

Productivity of under-ice algae was determined by a modification of the standard ^{14}C light and dark bottle technique (Strickland and Parsons 1972).

Ice and water samples were incubated *in situ* for 5-7 h periods, and ice algal biomass was estimated from chlorophyll *a* concentrations in the incubation chambers. Light was measured under the ice and used as a covariate in analyses of covariance (ANCOVA). Analysis of variance (ANOVA) and ANCOVA were used to examine field and laboratory techniques, temporal differences in biomass and productivity, and the effects of various levels of oil and dispersed oil on ice algal biomass and productivity.

Amphipods were captured and contained against undisturbed areas of the under-ice surface in cylindrical plexiglass chambers. Following exposures to different levels of oil for 3 and 15 h, and to the same levels of dispersed oil for 3 h, amphipods within the chambers were returned to the laboratory. Live and dead amphipods were separated, live amphipods were transferred to clean seawater, and live-dead separations were continued daily for 4 post-exposure days. Effects of oil level and exposure on behaviour and mortality were examined using correlation and ANOVA techniques.

Quantitative samples of amphipods were collected in the under-ice habitat at two times in May, in the shallow (3-5 m) sublittoral habitat in May, August and September, and in the intertidal habitat in August and September. Amphipods were identified, counted, weighed and measured. Spatial, seasonal and habitat variability in species composition, distribution and population structures were examined using ANOVA techniques.

Salinities and concentrations of nutrients (phosphate, nitrite, nitrate and silicate) at the productivity study site were typical of those found elsewhere in the Arctic, and sufficient to support the growth of sea ice

microalgae. Biomass and productivity of phytoplankton in the water immediately beneath the ice were low and typical for the season; biomass was 2 to 3 orders of magnitude lower than biomass of ice algae in the soft bottom layer of ice, and productivity values were near-zero. Ice algal biomass and productivity under control conditions were comparable to values reported elsewhere in the Arctic; biomass increased from about 6 to 11 mg Chl _a_/m^2 over the study period (16-30 May), and productivity ranged from 0.3 to 3.3 mg C/m^2/h at different times and in different locations in the 25-m-radius study area.

The highest oil level used (nominal concentration of 10,000 ppm) significantly suppressed algal productivity during each experiment, but in most cases productivity at lower levels (10 and 300 ppm) were not statistically distinguishable. In some cases, productivity at these lower oil levels was not different from productivity in control chambers. Results were consistent on the two days that untreated oil was used, but when dispersed oil was used, algal productivity was suppressed to a greater extent at the highest level (10,000 ppm) and there was some evidence of stimulation of photosynthesis at the lowest dispersed oil level (10 ppm). High productivity values in some dark (oiled) chambers and oil-related interference with measurement of chlorophyll and radiocarbon concentrations caused some interpretational difficulties.

Behavioural observations on oiled amphipods were performed _in situ_, and results refer to all amphipods. Most of the amphipods used in these experiments were later identified as _Weyprechtia pinguis_. Containment or handling effects were apparent both _in situ_ (crowding within chambers and availability of additional substrate) and in the laboratory, where mortality of control animals was sometimes high.

In the control chambers many amphipods were observed on the under-ice surface and in the water column. Prolonged exposure to oil resulted in the occurrence of a high proportion of these amphipods on the chamber bottoms (80-100% at the highest oil levels, 130 and 400 ppm). Impairment of locomotory ability by direct coating with oil may have been the cause. Degree of oiling (as measured on preserved amphipods) was directly related to mortality; only very lightly oiled amphipods survived for any length of time following exposure.

Immediate mortality of Weyprechtia pinguis (i.e. mortality at the end of the exposure period) increased significantly at higher levels of untreated oil. Immediate mortality was highest following the 15 h exposure to oil alone (58.5%), and delayed mortality was highest following the 3 h exposure to oil alone (40.6%). Total mortality was similar following 15 or 3 h exposures to undispersed oil (66.5, 68.6%), but much greater than total mortality after a 3 h exposure to dispersed oil at the same three levels. Total mortality after exposure to dispersed oil was less than half of that following exposure to oil alone.

The under-ice macrofauna in the three study bays included arctic cod (Boreogadus saida) and mysids (Mysis spp.), but otherwise consisted entirely of gammarid amphipods. Cod were observed only in the large tide cracks just inshore of the entry holes in each of the bays. Mysids were present throughout the water column in each bay, and were generally concentrated in the first metre of water just below the ice; densities were extremely high and variable both within and among bays.

Ten species of gammarid amphipods were collected on the under-ice surface at Cape Hatt. Two species, *Ischyrocerus* sp. and *Weyprechtia pinguis*, together accounted for 61.9% of total numbers, and three species, *Weyprechtia pinguis*, *Gammarus setosus* and *Onisimus litoralis*, together accounted for 86.1% of total biomass. By comparison, species diversity was considerably lower in the intertidal habitat (samples from August and September 1981) and considerably higher in the sublittoral habitat (samples from May, August and September 1981). Four amphipod species were found in the intertidal habitat; *Gammarus setosus* was dominant both in terms of numbers (93.2% of total) and biomass (76.8% of total). In the shallow (3-4 m) sublittoral habitat, 31 identified species and at least seven distinct but unidentified species were collected. *Orchomene minuta*, *Guernea* sp. and *Protomedia fasciata* together accounted for 50.7% of total numbers collected in May, August and September, and *Anonyx nugax*, *Anonyx sarsi*, *Orchomene minuta* and *Paroediceros lynceus* together accounted for 62.9% of total biomass.

All four species found in the intertidal habitat were also found on the under-ice surface. Three and seven of the 10 amphipod species found under the ice also occurred in samples from the shallow sublittoral habitat in spring and summer, respectively. Species occurring on the under-ice surface in May were of very little importance in the sublittoral habitat in May (0.3% of numbers, 1.1% of biomass), and of considerably higher importance in August and September (8.6% of numbers, 23.6% of biomass).

In general, abundance and biomass of all amphipods were highest in the sublittoral habitat, intermediate in the intertidal habitat, and lowest on the under-ice surface. Considerably fewer amphipods occurred on the under-ice surface in mid-May ($<15/m^2$) than in the sublittoral habitat at the same time ($>200/m^2$); differences in biomasses were less marked, but still very considerable. However, the species that dominated the under-ice habitat were more numerous there than on the bottom. Four dominant species, *Ischyrocerus* sp., *Weyprechtia pinguis*, *Onisimus litoralis* and *Gammarus setosus*, were present on the under-ice surface in relatively low abundances in mid-May. Of these, only *Ischyrocerus* and *Weyprechtia* were collected in the sublittoral habitat at that time, and then only in one bay. These data indicate a very strong preference for the under-ice habitat in May for the four dominant species on the under-ice surface. In sublittoral samples from August and September, *Gammarus* and *Ischyrocerus* were not present. *Weyprechtia* and *Onisimus* were absent or very rare in September.

Two cohorts (year classes) were apparent in length-frequency data for *Onisimus litoralis* and *Weyprechtia pinguis*, and at least three cohorts were present for *Gammarus setosus*. Growth (as estimated by mean size of a cohort) over the study period (May to August or September) was apparent for all species and cohorts. *Weyprechtia* was significantly variable in size among bays, but *Onisimus* and *Gammarus* were not. Juvenile *Gammarus* had apparently been released from brooding females before the study period began. Release of *Weyprechtia* occurred during the study period (17-31 May) and juvenile *Onisimus* were too few to warrant discussion.

In general, there were pronounced among-bay differences in abundances and biomasses of total amphipods and dominant species of amphipods on the under-ice surface. Furthermore, differences in abundance or biomass from one time to another (mid-May to late May, or August to September) were not consistent among bays; conversely, differences among bays were not consistent from one time to another.

Lagomedio crude oil was spilled as a surface slick in one bay between the August and September sampling periods, and a relatively even coating of oil was deposited on the beach (intertidal area) by the falling tide. Observations following the spill and results of the present study indicate that oil affected the amphipods that occupied the intertidal habitat. The numbers of intertidal *Gammarus setosus* decreased somewhat in dispersed oil bays from August to September, but the corresponding decrease in the bay contaminated with a surface slick was much more marked. *Onisimus litoralis* was absent or rare in the intertidal habitat in dispersed oil bays, but, like *Gammarus*, numbers decreased drastically in the intertidal area of the bay receiving the surface slick.

The absence or low abundance of *Gammarus* in the sublittoral habitat, and its abundance in the intertidal habitat, indicate that the latter is the source of recruitment for this species to the under-ice habitat. Thus, *Gammarus* may be less abundant on the ice in the bay contaminated with a surface slick in 1982 than in 1981. Sampling of all three habitats is continuing in 1982, and any effects of the summer spills on the under-ice communities in the following spring, as well as any recovery in the intertidal zone, should be particularly easy to detect for this species.

The results of the present study indicate that exposure to high levels of dispersed oil may suppress ice algal productivity more than exposure to the same (nominal) levels of untreated oil; procedural difficulties, however, render this conclusion tentative. Recovery of oiled algal communities subsequently exposed to clean water was not studied.

Amphipods exposed *in situ* to various concentrations of dispersed oil for a relatively short (3 h) period of time, however, were less affected than those exposed to oil masses together with low concentrations of dissolved oil components for relatively short (3 h) or long (15 h) periods of time. Amphipods in the intertidal habitat were apparently not affected by a large-scale dispersed oil spill, whereas mortality was high where untreated oil was spilled. These results indicate that the chemical dispersion of oil may be less harmful to amphipods in under-ice and intertidal communities than the accumulation of untreated oil in these habitats. Dispersed oil may contact much larger areas of the under-ice surface, however, resulting in greater total mortality. Further studies are required concerning the relative toxicities of dispersed and untreated oil at realistic concentrations, the recovery of communities exposed to oil, and, in particular, the behaviour of oil and dispersed oil under the ice.

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INTRODUCTION

In spring, a dense growth or bloom of microalgae occurs on and in the soft bottom layer of arctic sea ice. This algal layer begins to develop in April and the bloom peaks in May (Horner 1976, 1977). Productivity of ice algae during the relatively short bloom in April and May can be quite high. The bloom has been estimated to provide between 6 and 33% of the total annual primary production in various arctic locations (Alexander 1974; Horner et al. 1974; Welch and Kalff 1975). In addition, this bloom is important because its production occurs before there is significant production by planktonic and benthic algae during the open water season (Apollonio 1965). Thus, ice algal production is available to herbivores earlier in the season than is planktonic production (Dunbar 1968). This availability is further enhanced by the concentration of ice algae in two dimensions on the bottom of the ice and, near the end of the bloom, by their occurrence as macroscopic 'detrital' masses on the under-ice surface and in the water column (Cross 1982).

Ice algal concentrations are utilized by invertebrates occurring on the under-ice surface and in the water column (Bradstreet and Cross 1982). The largest and most conspicuous invertebrates inhabiting the under-ice surface are gammarid amphipods. Dominant species on the undersurface of the ice have included, at various places and times, Onisimus litoralis or O. glacialis (Green and Steele 1975; Buchanan et al. 1977; Thomson et al. 1978; Cross 1980, 1982; Grainger and Hsiao 1982), Gammarus setosus (Thomson et al. 1978), Ischyrocerus anguipes (Cross 1980, 1982) and Apherusa glacialis (Golikov and Scarlato 1973; Cross 1980, 1982). Most of these species are herbivores that consume ice algae (e.g., Horner and Alexander 1972; Buchanan et al. 1977; Bradstreet and Cross 1980, 1982); *O. litoralis* is also known as a scavenger of dead animal material (e.g., MacGinitie 1955). The habitats of these species in the absence of landfast ice include the undersurface of pan ice, the water column, and shallow sublittoral and intertidal areas. In the absence of ice, *Apherusa glacialis* and *Onisimus glacialis* are pelagic species (Dunbar 1954; Barnard 1959), although *A. glacialis* often associates with pan ice in late summer (e.g., Stephensen 1942; MacGinitie 1955; Divoky 1978; Thomson et al. 1978) and *O. glacialis* is also an epibenthic species in some locations (Griffiths and Dillinger 1981). *Onisimus litoralis* and *Gammarus setosus* occupy intertidal or nearshore sublittoral habitats in the open water season (Dunbar 1954; MacGinitie 1955; Steele and Steele 1970; Thomson and Cross 1980).

Distributional and dietary information indicates that the ice habitat is important to amphipods during spring. However, the relative importance and seasonal utilization of pelagic, benthic and under-ice habitats by these species is not known. Although the abundance and biomass of nearshore amphipods have been reported for intertidal, sublittoral and under-ice habitats in the eastern Arctic, simultaneous sampling of under-ice and benthic substrates and consecutive sampling of one area through the season (i.e. under fast ice and during the open water season) have not been carried out.

Ice-associated invertebrates are important food items for arctic cod (Bain and Sekerak 1978; Bradstreet and Cross 1980, 1982; Craig et al. 1982), various marine birds (Bradstreet 1976, 1980; Divoky 1978; Johnson and

Richardson 1981; Bradstreet and Cross 1982), and ringed seals (Finley 1978). In addition, the arctic cod is a major prey species of arctic marine mammals and birds in the Canadian Arctic (Dunbar 1941, 1949; McLaren 1958; Bradstreet 1976, 1977, 1979, 1980, 1982; Finley 1976; Davis and Finley 1979; Finley and Gibb 1982, in press; Finley et al. 1982) and elsewhere. Thus, under-ice communities may be critical elements of arctic marine food webs. Damage to the under-ice communities could have effects on the mammals and birds that occupy the higher trophic levels of the food webs.

Studies on plant and animal communities inhabiting the undersurface of arctic sea-ice, and in particular studies allowing direct observations by use of SCUBA methods, have become a focus of attention only recently. To date, few quantitative studies of this type have been conducted. Recent reviews of published research on under-ice biota in the Arctic and Antarctic are given by Horner (1976, 1977). These include details of research carried out by a group from the University of Alaska who used surface-operated and SCUBA methods to study microaglae and primary productivity during 1972-1974. SCUBA-based quantitative studies of under-ice communities in the central and eastern parts of the Canadian Arctic are those of Buchanan et al. (1977), Thomson et al. (1978) and Cross (1980, 1982).

In the event of a marine oil spill or blowout, large quantities of oil are most likely to accumulate in the under-ice, intertidal and shallow sublittoral habitats. Data on the effects of treated and untreated oil on the biota of these habitats would be of use in decisions regarding the use of chemical countermeasures for oilspills in ice-covered waters. Productivity and biomass of phytoplankton under oiled ice have been reported (e.g., Adams

1975), and laboratory experiments concerning effects of oil and dispersed oil on arctic phytoplankton have been carried out (e.g., Hsiao 1978), but similar studies of oil effects on ice algae have not been done. Laboratory studies concerning the acute toxicity of oil to arctic marine invertebrates have also been conducted, both in the Beaufort Sea (Percy 1974, 1976, 1977a,b; Percy and Mullin 1975, 1977; Busdosh and Atlas 1977) and in the eastern Arctic and sub-Arctic (Foy 1978, 1979). These studies have provided useful information on the relative sensitivities of a range of organisms, including some under-ice amphipod species, but laboratory studies cannot be used to predict the effects of oil contamination in a natural field situation (Foy 1978, 1979). _In situ_ studies of oil effects on nearshore arctic benthos were initiated in 1980 (Cross and Thomson 1981, 1982), but similar studies have not previously been carried out in arctic intertidal or under-ice habitats.

The present study examines effects of _in situ_ applications of oil and dispersed oil on the abundance and productivity of under-ice algae and on behaviour, mortality and distribution of under-ice amphipods. Immediate effects of oil on ice algae and amphipods were studied by injecting oil into small chambers on the under-ice surface. In addition, distribution and life history data were collected for amphipods occupying under-ice, intertidal and shallow sublittoral habitats. The latter component of the study utilizes the large-scale experimental spills carried out at the BIOS (Baffin Island Oil Spill) site in August 1981; we obtained pre-spill (May and August 1981) and post-spill (September 1981) data.

MATERIALS AND METHODS

Field studies were carried out during 16-31 May, 10-19 August and 7-8 September 1981 from the BIOS (Baffin Island Oil Spill) project base camp located at Cape Hatt, Baffin Island. The study area consisted of four shallow embayments in Ragged Channel, some 5-8 km SSE of Cape Hatt (72°27'N, 79°51'W). Bays 9 and 10 are shallow indentations in the coastline, each about 500 m in length, separated by the delta of a small stream and a distance of somewhat less than 500 m. Bay 13 is similar in size and configuration, located about 3 km to the north. Bay 11 has been designated as the lower half and Bay 12 as the upper half of a deeper embayment approximately 1 km x 1 km in dimensions, located approximately 1 km north of Bay 10 (Fig. 1).

All under-ice and sublittoral sampling and experimental work was carried out by SCUBA divers working though holes in the ice (May) or from small inflatable boats (August and September). Studies on amphipod distribution and population structure were conducted in Bays 9, 10 and 11 (Fig. 1), at a depth of 3-5 m or on the ice undersurface at the same locations. Intertidal sampling was carried out on the beach between two markers, 150 m apart, which demarcated the BIOS study bays. Experimental studies on amphipods and ice algae were conducted on the under-ice surface in Bays 12 and 13 over water depths of 8 and 12 m, respectively.

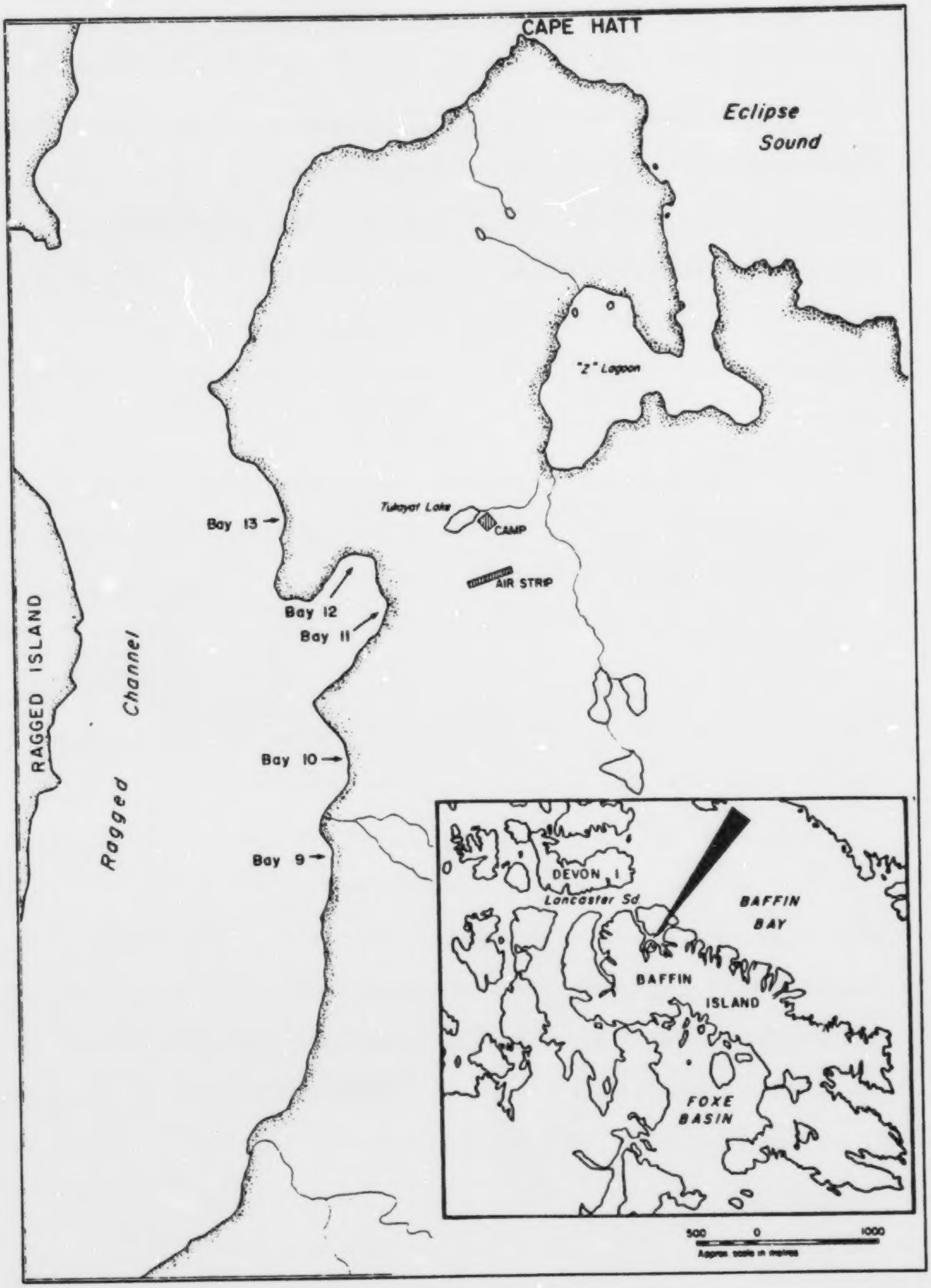
CAPE HATT
Eclipse Sound
"Z" Lagoon
Tukayat Lake
CAMP
AIR STRIP
Bay 13
Bay 12
Bay 11
Bay 10
Bay 9
RAGGED ISLAND
Ragged Channel
DEVON I
Lancaster Sd
BAFFIN BAY
BAFFIN ISLAND
FOXE BASIN
500 0 1000
Approx scale in metres

FIGURE 1. Locations of study bays at the BIOS project site at Cape Hatt, Baffin Island.

Ice Algal Biomass and Productivity

Productivity of under-ice algae in Bay 13 was determined by a modification of the standard ^{14}C light and dark bottle technique (Strickland and Parsons 1972). The 'bottles' in this case were cylindrical plexiglass chambers with an area of 20.27 cm^2 and a length of 15 cm (volume = 304 cc). Samples of ice algae were collected by inserting the chambers about 1-2 cm into the soft bottom layer of ice, severing the cores with a plexiglass spatula, and capping the chambers. Separate samples were collected in the same way for the determination of salinity, alkalinity and inorganic nutrient concentrations. Chambers to be used for productivity determinations were replaced immediately in their original positions, and ^{14}C-sodium bicarbonate (New England Nuclear Corp.) with a specific activity of 53 µCi/µmol was then injected to yield a final concentration of 50.0 µCi/L. Incubations began between 1200 and 1430 h and were allowed to proceed for a period of 5-7 hours. At the end of the incubation periods, 1 mL of concentrated formalin was injected and the chambers were returned to the field laboratory in insulated containers.

To avoid disturbance from air respired by divers, samples were collected, and chambers replaced, in rows along ridges on the under-ice surface. Adjacent chambers were about 20 cm apart. Light was measured with a photometer (InterOcean model 510) at each end of the row of chambers below the layer of ice algae, and above the algal layer, after scraping this layer away. These measurements were made at the beginning and end of each incubation, and simultaneous measurements above the ice were made with a surface cell so percent transmission through the ice could be calculated. A recording pyranometer (Kipp and Zonen, model CM-6) located at the Cape Hatt base camp recorded incoming radiation (W/m^2) during the 4 weeks that the camp was occupied.

Three types of experiments were carried out:

1. Control Measurements. On each of 16 and 21 May, a set of nine chambers was placed in each of two locations within a 25 m radius of the dive hole (total of 4 sets of 9 chambers). Each set consisted of five light and two dark chambers including water and ice as described above, and one light and one dark chamber including water only. The 'water only' chambers were collected immediately below the undersurface of the ice. Further control measurements were available from the other types of experiments (below).

2. Oil Effect Experiments. The effects of untreated oil were studied on 23 and 24 May, and dispersed oil effects were examined on 30 May. Unweathered Lagomedio crude oil (Esso Resources Canada Ltd.), and a 10:1 mixture of Lagomedio : Corexit 9527 (Exxon Chemical Corp.) were the types of oil and dispersed oil used. Three oil levels (nominal concentrations of ~ 10 ppm, 300 ppm and 10,000 ppm) and a control were used on each day; one dark and three light chambers were used for each oil level (including control). Each chamber was numbered and the same chambers were used for the same oil levels on each day. Oil was injected into the chambers before the injection of ^{14}C-bicarbonate.

3. Test of experimental technique. On 29 May a test was made to determine the effectiveness of the chambers in containing the ^{14}C-bicarbonate

solution, and the effect of detaching the ice algal layer from the under-ice surface prior to incubation. Samples were collected, and incubations carried out, in six light chambers as described above under control measurements. An additional six light chambers from the same location were treated identically except that the ice cores contained within the chambers were not severed, and the tops of the chambers were not capped, until the end of the incubation period.

Carbonate alkalinity was calculated according to the methods of Strickland and Parsons (1972). A Fisher Accumet pH meter (model 630, accuracy ± 0.02 pH) was used for the measurement of pH, and salinity was calculated from Knudsen tables using temperature and specific gravity measurements obtained with a hydrometer (Fisher, 1.000-1.070).

Ice in samples from incubation chambers was allowed to melt at room temperature; unoiled samples were then stirred thoroughly, and subsampled for ^{14}C (50 mL) and chlorophyll (100 mL) determinations. All samples were filtered under a vacuum pressure of 200 mm Hg. ^{14}C samples were filtered through 0.45 μm Metricel membrane filters; the filters were then rinsed twice with 15 mL filtered seawater and placed in 20 mL Aquafluor (NEN Corp.) in borosilicate glass scintillation vials. Chlorophyll samples were filtered through 0.7 μm Whatman glass microfibre filters (GF/F), with a few drops of $MgCO_3$ added at the end of filtration. The filters were placed in glassine envelopes and frozen in plastic bags containing silica gel.

The contents of incubation chambers containing oil were, after melting and stirring, poured into 500 mL pear-shaped separatory funnels and allowed to stand for 1/2 h. The lower 300 mL were then withdrawn and treated as for control chambers. Chambers containing dispersed oil were treated in the same way except that those containing the highest oil level were allowed to stand in the separatory funnels for 12 h. The effect of this procedure (use of separatory funnels to separate oil from water) was tested as follows: After subsampling control chambers for the 'experimental technique' test (above), the remaining contents of the 12 chambers were combined and diluted with filtered seawater to form a uniformly labelled stock mixture. Sixteen incubation chambers were filled with this solution, and oil was injected as in the *in situ* incubations (i.e. 4 chambers of each of 0, 10, 300, and 10,000 ppm). The chambers were then processed as for the *in situ* incubations (separatory funnels, filtration) except that 100 mL was filtered for ^{14}C samples.

^{14}C radioactivity was measured at the Arctic Biological Station at Ste-Anne-de-Bellevue, Quebec, using a Nuclear Chicago Isocap 300 Scintillation counter; counting inefficiencies were corrected by using the channel ratios method. Chlorophyll *a* concentrations were determined using a Turner model III Fluorometer at excitation and emission wavelengths of 430 nm and 630 nm, respectively (A.P.H.A. 1975). Nutrient concentrations were determined using a dual beam Beckman Acta 3 spectrophotometer (A.P.H.A. 1975). Chlorophyll and nutrient concentrations were measured at Guelph Chemical Laboratories Ltd., Guelph, Ontario.

Analyses of variance (ANOVA) and covariance (ANCOVA) were performed by the SAS general linear models (GLM) program (Helwig and Council 1979). In analyses of covariance, light level (W/m^2) during the experiment was used as a covariate for productivity ($mgC/m^2/h$), and percent transmission was used as a covariate for biomass (mg chlorophyll a/m^2). Light levels were estimated from solar radiation at the Cape Hatt camp and percent transmission through the ice at the location of each experimental chamber. Percent transmission was calculated as 100 times the average of the light readings below and above the ice algal layer, divided by the reading at the upper surface of the ice. Percent transmission for each replicate (chamber) was estimated by linear interpolation between the measured values at each end of the row of chambers. Solar radiation falling during 1/2-h periods through the incubations was calculated by planimetry from the recording pyranometer records. These figures were multiplied by the percent transmission during these 1/2-h periods (assuming a linear change in percent transmission with time of day) to give *in situ* irradiance. ANOVA and ANCOVA were supplemented with multiple comparisons (Duncan's multiple range test, $\alpha = 0.05$).

Oil Effects on Under-ice Amphipods

Amphipods were captured and contained against undisturbed areas of the under-ice surface in cylindrical plexiglass chambers with an area of 188.7 cm^2 and a length of 20 cm (volume = 3774 cc). Air-filled plexiglass collars around the chambers held them in place against the under-ice surface. The bottoms of the chambers were covered with 1 mm mesh netting with a centrally located valve through which oil was injected.

Three types of exposure to oil were tested: On 26 and 27 May, chambers containing unweathered Lagomedio crude oil were left in place for 15 hours and 3 hours, respectively. On 28 May, chambers containing dispersed oil (10 Lagomedio:1 Corexit 9527) were left in place for 3 hours. On each day, three oil levels and a control were used; three replicate chambers were used for each oil level (including control). Volumes of 0.1, 0.5 and 1.5 mL of oil were used to give nominal concentrations of approximately 30, 130 and 400 ppm. Oil, dispersed oil, or water (controls) was introduced through the chamber bottoms by vigorously pumping a 3 mL syringe ten times. Oil/Corexit mixtures became evenly dispersed throughout the chambers and persisted during the three hour exposure. In oil alone treatments, droplets rose to the ice and persisted in the form of small, evenly distributed spheres. Droplet size increased from approximately 0.5 to 2 mm in diameter with increasing oil level, and at all levels the water within the chambers became noticeably discoloured. Each chamber was numbered, and the same chambers were used for the same oil levels on each day.

The numbers of amphipods on the ice, in the water and on the bottom of the chamber were recorded before injection, immediately after injection, and at the end of the incubation period. These observations were possible only for exposures to oil alone, as the reduced visibility within dispersed oil chambers hindered or precluded observations. After the last set of observations, the ice core contained within the chamber was severed with a plexiglass spatula, and the chamber was capped. Chambers were returned to the field laboratory in insulated containers.

Upon arrival at the field laboratory, amphipods and ice from each chamber were transferred into clean seawater (~ 33 $^{o}/oo$) in large trays. Live and dead animals were separated, the criterion for death being failure to move when prodded. Dead animals were transferred into 5% formalin, and live animals were placed in glass jars of seawater and maintained at 0°C in incubators. The animals were checked, and live-dead separations made at 24-h intervals thereafter until 96 h after exposure, at which time live amphipods were transferred into separate vials containing 5% formalin. Thus each replicate (chamber) produced a maximum of 6 preserved samples: those dead on arrival (day 0), those dying on 4 successive days (days 1-4), and those surviving (day 5).

Subsequent laboratory analysis was carried out within one month. For each amphipod in each sample, the following data were recorded (where possible): species, sex, length (mm), wet weight (mg), and degree of oiling (light, medium, heavy) on each of eight body areas: coxae, pleopods, mouth parts, head/peraeon, pleon, urosome/uropods/telson, gnathopods/peraeopods, and antennae.

Behavioural data were analyzed using the non-parametric Spearman rank correlation method (hand computation). All other data were coded for computer processing and analyzed using SAS programs (Helwig and Council 1979). Variation among oil levels and exposures in percent mortality, both immediate and delayed, and in percent survival, was analyzed by two-factor ANOVA (oil level x exposure) and separate one-factor ANOVA's for both abundance and biomass data. The difference in mean size between all amphipods in a chamber and those dying was calculated where sample sizes were ≥ 3, and among-treatment differences in these values were analyzed by two-factor ANOVA. Contingency tables were compiled for degree of oiling *vs.* day of death for each amphipod in oil alone treatment chambers (excluding controls), and a X^2 value was computed.

Under-ice Amphipod Distributions and Population Structures

Quantitative samples of amphipods were collected in under-ice, intertidal and shallow (3-5 m) sublittoral habitats in each of BIOS study Bays 9, 10 and 11 (Fig. 1). The under-ice surface was sampled at two times, on 17-19 May and 31 May 1981 (10-16 replicates per bay per time). The intertidal habitat was also sampled at two times, on 17-19 August and on 7 September 1981, and the shallow sublittoral habitat was sampled at three times, on 17-19 May, 10 August and 8 September 1981 (10 replicates per habitat per bay per time). In late August 1981, 15 m^3 of untreated Lagomedio oil was released within booms on the surface of Bay 11, and an additional 15 m^3 of the same oil treated with the dispersant Corexit 9527 (10 oil:1 Corexit) was released underwater in Bay 9 (Fig. 1). Currents carried the dispersed oil into Bay 10, which had originally been designated as the control bay. This resulted in a relatively high level of contamination of Bay 10--approximately one order of magnitude lower than that in the dispersed oil spill bay.

All systematic sampling on the under-ice surface was at least 5 m from the entry hole to avoid disturbance artifacts. Macrofauna (mainly amphipods) on the under-ice surface were sampled by scraping fine mesh (1 mm) dip nets with a 40 cm flat top for 10 m distances along the under-ice surface in areas not previously disturbed by respired SCUBA air. These 10 m transects extended radially from the entry hole (5-15 m, 15-25 m) and were relatively evenly distributed in the semi-circle seaward of the hole. Entry holes were located just seaward of major tide cracks, and the area sampled consisted only of relatively flat, smooth ice.

Intertidal sampling was carried out at or near low tide. A 0.25 m^2 aluminum quadrat was placed in the water 0.5 m seaward from the water line, the substrate was manually disturbed, and all of the enclosed animals were removed by using a small aquarium net. Sampling locations were randomly selected along 150 m segments of the bays that corresponded with the BIOS study benthic transects. Substrates consisted of mixed sand, pebble and cobble.

Sublittoral sampling was carried out using a self-contained diver-operated airlift. The airlift consisted of a weighted length of pipe 8 cm in diameter fitted at the top with a 1 mm mesh net, which retained the sample and could be removed quickly and capped. Air was supplied from a 20 MPa air cylinder fitted with the first stage of a diving regulator which reduced air pressure to approximately 860 kPa above ambient. Areas to be sampled were demarcated by an aluminum ring containing an area of 0.15 m^2. Motile epibenthos within the 0.15 m^2 area were contained, and those outside were excluded, by 1 mm mesh netting covering the top of each ring. The netting over each ring contained a capped central receptacle to receive the 'mouth' of the airlift.

The airlift frame was placed on the bottom and pushed as far as possible into the substrate to contain shallow infauna. The airlift was attached to the net, the air was turned on, and the mouth of the airlift was moved around to cover thoroughly the area within the ring. The net on the airlift was then removed, capped and replaced.

In May, sublittoral sampling locations were randomly selected at a depth of 3-5 m within an area on the bottom below the under-ice sampling area. In August and September, samples of epibenthic and shallow infaunal amphipods were again collected at the same depths, but in areas only approximately corresponding to those sampled in May. The substrate consisted of coarse to fine sand with pebbles and cobble (up to 10 cm).

All samples were preserved in 10% formalin. Amphipods were identified, counted and weighed at species level (whenever possible) and amphipod lengths were measured to the nearest mm. Amphipods of the genus Onisimus were not identified to species if <6 mm long; most Ischyrocerus collected were damaged and hence were only identified to generic level. Wet weights were obtained by gently blotting dry and weighing on a Mettler PT200 balance to the nearest milligram.

The resulting data were analyzed with one- and two-factor analyses of variance, using the SAS general linear models (GLM) program (Helwig and Council 1979). Variables analyzed included abundance (nos./m^2) and biomass (mg/m^2) of all amphipods and of dominant species, and, only for dominant species, mean size of each cohort in each sample where n (in a cohort) >3. Cohorts (year classes) were identified from size-frequency plots for each species for each month. All data were log-transformed prior to analysis.

RESULTS AND DISCUSSION

Ice Algal Biomass and Productivity

Site Description

The under-ice surface in the study area was smooth and relatively flat, with shallow hummocks and ridges. Productivity studies were carried out in these areas of thicker ice, and ice depth was, therefore, somewhat greater than the measured depth of 135 cm at the entry hole. Snow depths over the study area (a semi-circle 25 m in radius) were 15.8 ± SD 5.1 cm, 19.5 ± 7.2 cm and 17.2 ± 5.4 cm on 16 May, 21 May and 1 June, respectively (n = 24-26 in each case). The amount of light penetrating the snow and ice cover in the study area varied both spatially (primarily because of variable snow cover), and temporally; temporal variation, within and among days, resulted from changes in cloud conditions and in solar elevation. Total *in situ* radiation varied among incubation periods by almost an order of magnitude.

Salinity of ice and ice/water samples ranged from 30.8 to 33.6 $^{o}/oo$; no consistent differences were apparent either between water and ice/water samples, or among days on which determinations were made (16-30 May). The number of stalactites on the undersurface of the ice increased over the study period, probably indicating increased drainage caused by increased surface temperature. Snow melt was also beginning near the end of May, but no obvious effects were observed under the ice. A thin (several cm) fresh water layer was observed immediately beneath the ice in other bays, but within the productivity study area no such layer was evident.

Nutrient samples were collected in duplicate on 21, 23, 24, 29 and 30 May. Phosphate concentrations were somewhat higher on 23 May (4.86 and 18.23 μmol/L) than on other days (1.31 to 3.19 μmol/L), whereas nitrate concentrations were lower on 21 and 23 May (<0.01 to 1.81 μmol/L) than on the following days (4.13 to 6.01 μmol/L). Nitrite and silicate concentrations were relatively constant over the study period (<0.04 to 0.08 μmol/L and 6.12 to 14.45 μmol/L, respectively). Consistent differences between water and ice + water samples were not evident for any nutrient. With the exception of the high phosphate concentrations on 23 May, which may indicate contamination, nutrient concentrations fall within ranges previously reported (e.g., Alexander et al. 1974; Cross 1980, 1982; Grainger and Hsiao 1982), and are sufficient to support the growth of ice microalgae (Hsiao 1980).

Phytoplankton Biomass and Productivity

Biomass (as estimated by chlorophyll a concentration) and productivity were very low in the water immediately beneath the ice on 16 and 21 May. The concentration of chlorophyll a in water samples (1.19 ± SD 0.47 mg/m^3; n = 8) was similar to that reported in other locations (Alexander et al. 1974; Cross 1980, 1982; Grainger and Hsiao 1982). After correction for dilution (sampled ice depth = 1 to 2 cm; chamber depth = 15 cm), the algal biomass per unit volume was lower in the water than in the ice by 2 to 3 orders of magnitude.

Productivity in the near-ice water was also low; indeed, after dark ^{14}C uptake (0.28 ± 0.09 mg C/m^3/h; n = 4) was subtracted from light ^{14}C fixation, productivity values were slightly but consistently negative (-0.033 ± 0.023 mg C/m^3/h; n = 4). Alexander et al. (1974) also reported low productivity estimates for phytoplankton in May at Barrow, Alaska: averages of 0.30 and 0.23 mg $C/m^3/h$ for 1972 and 1973, respectively.

Ice Algal Biomass and Productivity

Ice algal biomass, as estimated by chlorophyll content in the ice, increased significantly over the study period (ANOVA; F = 5.1; df = 5, 45; P = 0.0009). Mean values (± SD) ranged from 6.56 ± 1.95 mg Chl*a*/m^2 (n = 14) on 16 May to 10.93 ± 0.64 mg Chl*a*/m^2 (n = 4) on 30 May. These data are comparable to those of Dunbar and Acreman (1980) for two arctic locations in May, and those of Clasby et al. (1973) for Barrow, Alaska, in late May, but are considerably lower than chlorophyll concentrations at fast ice stations in Pond Inlet during May and June reported by Cross (1982), *viz* 17.6 to 182.6 mg Chl*a*/m^2. This probably is attributable to the relatively deep snow present at the Cape Hatt study area; the lowest mean biomass reported for Pond Inlet (above) was at the station with the highest mean snow depth (15.8 cm), and a significant negative correlation between snow depth and biomass was found at some stations (Cross 1982). An inverse relationship between ice chlorophyll and snow depth has also been observed by Alexander et al. (1974).

Based on the data above, the net increase in epontic algal biomass was 4.37 mg Chl*a*/m^2 for the two week period, or 0.31 mg Chl*a*/m^2/d.

Mean ice algal productivity on any day in any location (control data only) ranged from 0.26 ± SD 0.07 mg $C/m^2/h$ (n = 5) to 3.34 ± 1.96 mg $C/m^2/h$ (n = 5); the overall mean for the study period was 1.83 ± 1.39 mg $C/m^2/h$

(n = 35). When total productivity during incubation periods was compared with the amount of _in situ_ radiation present during the incubations on a day/location basis, the correlation was significant and positive ($r = 0.87$, $n = 8$, $P<0.01$). When productivity data from different day/location combinations were compared using ANOVA, differences were significant ($F = 5.71$; $df = 7, 26$; $P<0.001$); however, when light data were used as a covariate (ANCOVA), the day/location differences became non-significant ($F = 1.73$; $df = 7, 26$; $P = 0.146$).

These data are comparable to those of Clasby et al. (1973) at Barrow, Alaska, who reported mean productivity values between 4 and 4.5 mg $C/m^2/h$ on 6 and 21 May, and values between 1 and 1.5 mg $C/m^2/h$ between 25 May and 5 June. It appears that the under-ice algal bloom, and concomitant high productivity, had begun to decline earlier at Barrow in 1972 than at Cape Hatt in 1981.

The increase in biomass from 16 to 30 May (see above) can be compared with our productivity estimates. Using a C/Chl_a_ ratio of from 23 to 79 (Parsons et al. 1977, Table 11), and the daily rate of Chl_a_ increase calculated above, the net amount of carbon fixed per day during the study period was 7.1 to 24.5 mg C. Total ^{14}C productivity during 5 to 7 h incubation periods ranged from 1.5 to 17.6 mg C/m^2 (overall average of 10.7 mg C/m^2). Assuming that productivity continues at this rate from 12 to 24 h per day (the latter is not likely), average daily productivity would be about 20 to 40 mg C/m^2. Admittedly these are very rough calculations, but it is evident that the two methods provide estimates that are at least within the same order of magnitude. A refinement of this productivity estimate would require, at the very least, productivity measurements during the arctic 'night' in spring, and these are presently not available.

Two methods of collecting microalgal samples for incubations were compared: (1) inserting the chambers, cutting the core, and capping before ^{14}C injection and incubation, and (2) inserting the chamber before ^{14}C injection but cutting and capping after the incubation. Biomass of epontic algae did not differ significantly between the two methods (ANOVA, F = 1.53; df = 1, 9; P = 0.247). Productivity, however, was significantly higher with the second method (2.89 ± 0.44 mg C/m^2/h) than with the first (1.58 ± 0.46 mg C/m^2/h) according to ANOVA, and, more appropriately, ANCOVA with light as the covariate (F = 16.99; df = 1, 9; P = 0.0026). This probably was attributable to disturbance of the epontic algae when the core was cut prior to incubation. If there was any leakage of ^{14}C-bicarbonate when we used the second method (not strictly a closed system), then this disturbance effect would be even greater than our data indicate. The second method was the one used by Clasby et al. (1973), and clearly is the preferred method. The first method was the one used in the rest of our experiments, and hence our productivity estimates are low by a factor of approximately two.

Oil Effects

Laboratory Techniques

Oil or dispersed oil was injected into the incubation chambers at the start of incubation with ^{14}C, and remained there throughout the incubation periods. Previous investigators studying oil effects on productivity have not discussed effects of oil on laboratory techniques; this is a concern because of possible quenching effects on scintillation counts and interference in the fluorometric determination of chlorophyll concentrations. *A priori* tests of the former indicated that only small amounts of oil could be tolerated in scintillation cocktails, and therefore we attempted to separate the oil from the incubation medium before filtration (see 'Methods'). Interference by oil in fluorometry was not investigated.

In order to determine whether the separation of oil and incubation medium had any effect on ^{14}C activity or apparent chlorophyll concentrations, we performed a separate test using a uniformly labelled stock to which the three oil levels were added (see 'Methods'). Specifically, it was suspected that labelled algae may have remained with the oil in the separatory funnels. The results of this test were as follows:

Nominal Oil level (ppm)	^{14}C Activity (dpm)	Chlorophyll *a* (mg/m^3)
	mean ± SD (n = 4)	mean ± SD (n = 4)
0	7255.5 ± 234.34	25.11 ± 3.66
10	6098.5 ± 131.58	20.18 ± 0.90
300	4069.0 ± 680.60	17.20 ± 3.51
10,000	3665.3 ± 381.63	15.25 ± 0.73

Results of ANOVA showed that the decrease with increasing oil level was significant, both for chlorophyll *a* and for ^{14}C activity. Correction factors were then calculated for each variable, and applied to the results of the *in situ* incubations (23, 24 May) using the same oil levels. A similar test was not conducted using dispersed oil, and so those data were not corrected.

A comparison of the results for oil incubations using corrected and uncorrected data, however, indicated that the _in situ_ addition of oil did not have the same effect as did the laboratory addition of oil. ANCOVA on uncorrected biomass (Chl _a_) data showed non-significant variation among oil levels, both on 23 May (F = 2.29; df = 3, 11; P = 0.14) and on 24 May (F = 0.77; df = 3, 11; P = 0.54). The same analyses using 'corrected' biomass data showed marginally significant differences among oil levels on 23 May (F = 3.71; df = 3, 11; P = 0.046) and highly significant differences on 24 May (F = 6.57; df = 3, 11; P = 0.008). Moreover, the differences in corrected data were not those expected--on both days higher corrected biomass values were calculated for the two higher oil levels than for the control or the lowest oil level. A possible explanation is that, in the laboratory experiment, algae in chambers were dispersed throughout the water column, and hence more susceptible to entrainment by the injected oil than were the algae in the _in situ_ experiments, which were concentrated in the ice.

Another probable artifact became apparent only after inspection of the scintillation counts. In most cases, dark ^{14}C uptake in oiled incubation chambers (169 to 606 dpm) was within the range of dark ^{14}C uptake values measured in control chambers. Relatively high values, however, were recorded in some of the dark chambers to which oil was added: 1792 dpm (23 May, 300 ppm); 2266 dpm (24 May, 10,000 ppm); 13,168 dpm (30 May, 100 ppm); 1751 dpm (30 May, 300 ppm). Dark values were routinely subtracted from each of the corresponding replicate light values in the calculation of productivity to account for dark ^{14}C uptake by algae; high dark uptake from other sources (biotic or abiotic) would lead to significant underestimates of algal productivity. Thus, we present productivity data (below) based on (1) measured dark values at each oil level on each day, and (2) control dark values for each day, applied to all oil levels.

Ice Algal Productivity

Data on the effects of oil and dispersed oil on ice algal productivity are given in Table 1. The results of 3 methods of calculation are included: data corrected for ^{14}C loss resulting from the separation of oil from the incubation medium prior to filtration (see above), and uncorrected data using dark incubation values from (1) chambers containing oil and (2) control chambers (see also above). The productivity values vary among calculation methods, but the results of ANCOVA are similar for all methods.

High oil levels suppressed productivity significantly on each day, regardless of the method of calculation used. One-factor ANCOVA with oil level as the factor and light as the covariate revealed a significant ($P<0.01$) oil effect in every case. In all cases, productivity was lower at the highest oil level than at any other level including controls (Table 1). Results of multiple comparisons, however, showed that differences among, 0, 10 and 300 ppm oil levels were not consistent either among days or types of data. In most cases productivity at the lowest levels (10 and 300 ppm, or 0, 10 and 300 ppm) were not statistically distinguishable (Table 1).

Two-factor ANCOVA for the two days when untreated oil was used showed significant variation among oil levels ($P<0.001$), but no significant variation between days ($P>0.07$) and no significant interaction between days and oil levels ($P>0.65$). This was true regardless of which of the three

Table 1. Effects of oil and dispersed oil on the productivity (mg C/m^2/h) of under-ice algae at Cape Hatt, Baffin Island, during May 1981. Data shown are mean ± SD (n = 3) for each date and oil level; results are shown for 3 different methods of calculation.

		Ice Algal Productivity (mg C/m^2/h)		
		Uncorrected Data[2]		
Date[1]	Nominal Oil Level (ppm)	Oil level dark values	Control dark values	Data corrected for oil/medium separation[2]
23 May	0	1.92 ± 0.43	1.92 ± 0.43	1.92 ± 0.43
	10	2.17 ± 0.28	2.19 ± 0.28	2.58 ± 0.33
	300	1.32 ± 0.13	1.47 ± 0.13	2.36 ± 0.23
	10,000	0.38 ± 0.18	0.36 ± 0.18	0.76 ± 0.35
	Multiple Comparisons[3]	3<2<0,1	3<0,1,2; 2<1	3<0,1,2
24 May	0	2.21 ± 0.33	2.21 ± 0.33	2.21 ± 0.33
	10	0.82 ± 0.22	0.81 ± 0.22	0.97 ± 0.26
	300	0.74 ± 0.07	0.73 ± 0.07	1.31 ± 0.12
	10,000	0.13 ± 0.13	0.34 ± 0.13	0.26 ± 0.26
	Multiple Comparisons[3]	3<1,2<0	3<0,1; 1,2<0	3<1,2<0
30 May	0	1.64 ± 0.21	1.64 ± 0.21	-
	10	0.88 ± 0.21	2.63 ± 0.21	-
	300	0.61 ± 0.15	0.81 ± 0.15	-
	10,000	0.01 ± 0.01	-0.01 ± 0.01	-
	Multiple Comparisons[3]	3<1,2<0	3<2<0<1	-

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[1] Untreated Lagomedio crude oil was used on 23 and 24 May; 10 Lagomedio:1 Corexit 9527 was used on 30 May.

[2] See text for methods of calculation.

[3] Duncans multiple range test, α = 0.05; codes 0-3 represent nominal oil levels 0, 10, 300 and 10,000 ppm, respectively.

methods of calculation were used. Thus, results were consistent on the two days when untreated oil was used.

The effects of dispersed oil on ice algal productivity appeared to differ from those of untreated oil in two ways. (1) The highest oil level (10,000 ppm) resulted in near zero productivity in the case of dispersed oil, whereas it resulted in much reduced but still positive productivity in the case of untreated oil. Laboratory treatment of the highest dispersed oil level, however, was unique (longer time in separatory funnels), so it is uncertain whether the greater apparent decrease in productivity was real. (2) The only significant increase in productivity with increasing oil level occurred on 30 May (dispersed oil day): productivity was 60% greater at 10 ppm dispersed oil than in controls (Table 1), but only when control dark values were used. Hsiao et al. (1978) presented some evidence of a stimulation of photosynthesis when 'dispersed oil' (10 ppm Pembina crude oil + 10 ppm Corexit) was added to natural arctic phytoplankton communities, but it is not clear whether their results are based on dark bottles containing dispersed oil. In the absence of an adequate explanation for the high dark values observed within oiled chambers in the present study, the validity of the observed stimulation of productivity cannot be assessed.

Despite the procedural and interpretational difficulties discussed above, it is clear that the highest oil level studied had a pronounced effect on ice algal productivity. At this level, dispersed oil apparently had a larger effect (total suppression of productivity) than did undispersed oil, but even in the latter case productivity was much reduced from control levels. Nominal concentrations of oil and dispersed oil were the same at each level; actual concentrations were not measured but were undoubtedly much lower in the case of undispersed oil. Hsiao et al. (1978) reported oil effects on productivity of arctic marine phytoplankton in oil-seawater dispersions at concentrations as low as 10 ppm; at concentrations between 43 and 147 ppm values were 20 to 40% of control productivity, depending on type of oil. Similar results on diatom growth were obtained when oil was added directly to algal cultures to give nominal concentrations between 10 and 10,000 ppm (Hsiao 1978). After 10 day's exposure, diatom growth was reduced at all concentrations for all oils tested, but the highest concentration was lethal only to some combinations of species and oils. Further studies are required in order to determine if the observed effects on growth and productivity are reversible, particularly in the case of chemically dispersed oil which would likely only contact the ice for a limited period of time.

Oil Effects on Under-ice Amphipods

Site Description

Amphipod experiments were carried out in Bay 12 (Fig. 1) over a water depth of approximately 8 m. Ice depth at the entry hole was 156 cm, and snow depth in the study area (a circle of 25 m radius) was 26.2 ± SD 5.6 cm (range of 12-38 cm; n = 40). A layer of fresh water several cm thick was observed just beneath the ice on each of the study days (25-28 May). Amphipods were collected in the incubation chambers and the chambers were then placed on shallow hummocks or ridges on the under-ice surface. In this way we avoided both disturbance of the under-ice surface by respired air from SCUBA and any effects of the fresh water layer occurring under thinner ice.

The most abundant amphipod in the study area, and hence in the experimental chambers, was Weyprechtia pinguis (76.5% of total number in chambers). The remaining 23.5% of the total number included 7 distinct species as well as unidentified damaged and juvenile amphipods. One or more of the chambers at each oil level on 26 May contained only a few amphipods (2 to 11), so for that day the results for two chambers at each oil level were pooled to provide adequate numbers for analysis (behavioural data excluded). All results below are concerned only with Weyprechtia (except for behaviour, when amphipods were not identified), as numbers of other species were too low to allow meaningful analysis.

Behaviour

Most of the amphipods in the study area occurred on the undersurface of the ice; when disturbed, amphipods either dropped, motionless, through the water column, or began swimming downwards. In either case, upward motion began within a few seconds and the amphipods resumed their positions on the ice, sometimes leaving and returning to the ice several times before settling. Similar behaviour was observed within incubation chambers, although containment effects were also apparent. Of the enclosed amphipods, only half were on the under-ice surface prior to oiling, and the rest were distributed evenly between the water column and the chamber bottoms (averages of 50.2% on ice, 26.0% in water, and 23.8% on bottom; all chambers included). The lower proportion of amphipods on the ice relative to that outside of the chambers (close to 100%) probably was attributable to the availability of another surface (chamber bottom) or to crowding on the ice within the chambers. When oil, dispersed oil or water was injected through the chamber bottom, most amphipods left the ice and swam around the chamber and upwards towards the ice; a prolonged setting process was again observed. Approximately five minutes after 'oiling', fewer amphipods were on the under-ice surface, and more were in the water and on the chamber bottoms than prior to 'oiling' (averages of 31.0% on ice, 39.7% in water and 29.3% on bottom; dispersed oil chambers not included).

During the observation period there was no apparent avoidance of oil by amphipods; in fact, direct contact between amphipods and oil droplets was often observed while amphipods were settling on the under-ice surface. We observed no obvious differences in amphipod behaviour among oil levels (controls included), and the proportions of amphipods on the under-ice surface before or shortly after oiling were not correlated with oil level during any experiment (Spearman rank correlation, maximum $r_s = -0.40$ for $n = 11$). 'Abnormal' behaviour, i.e. circular swimming, prolonged settling time and reduced numbers of amphipods on the ice, occurred in both control and treatment chambers and hence must have been the result of confinement in the relatively small chambers or disturbance due to the injection of oil or water.

Prolonged exposure to oil was the only factor that markedly affected the percentage of the amphipods that dropped to the bottom of the chamber. Occurrence of amphipods on the chamber bottoms was not significantly correlated with oil level either before or immediately after the injection of oil, during either exposure period (Fig. 2). In most cases, more than 50% of the amphipods were in the water column or on the ice. At the end of the 3 h exposure period, amphipod distribution in control chambers was similar to

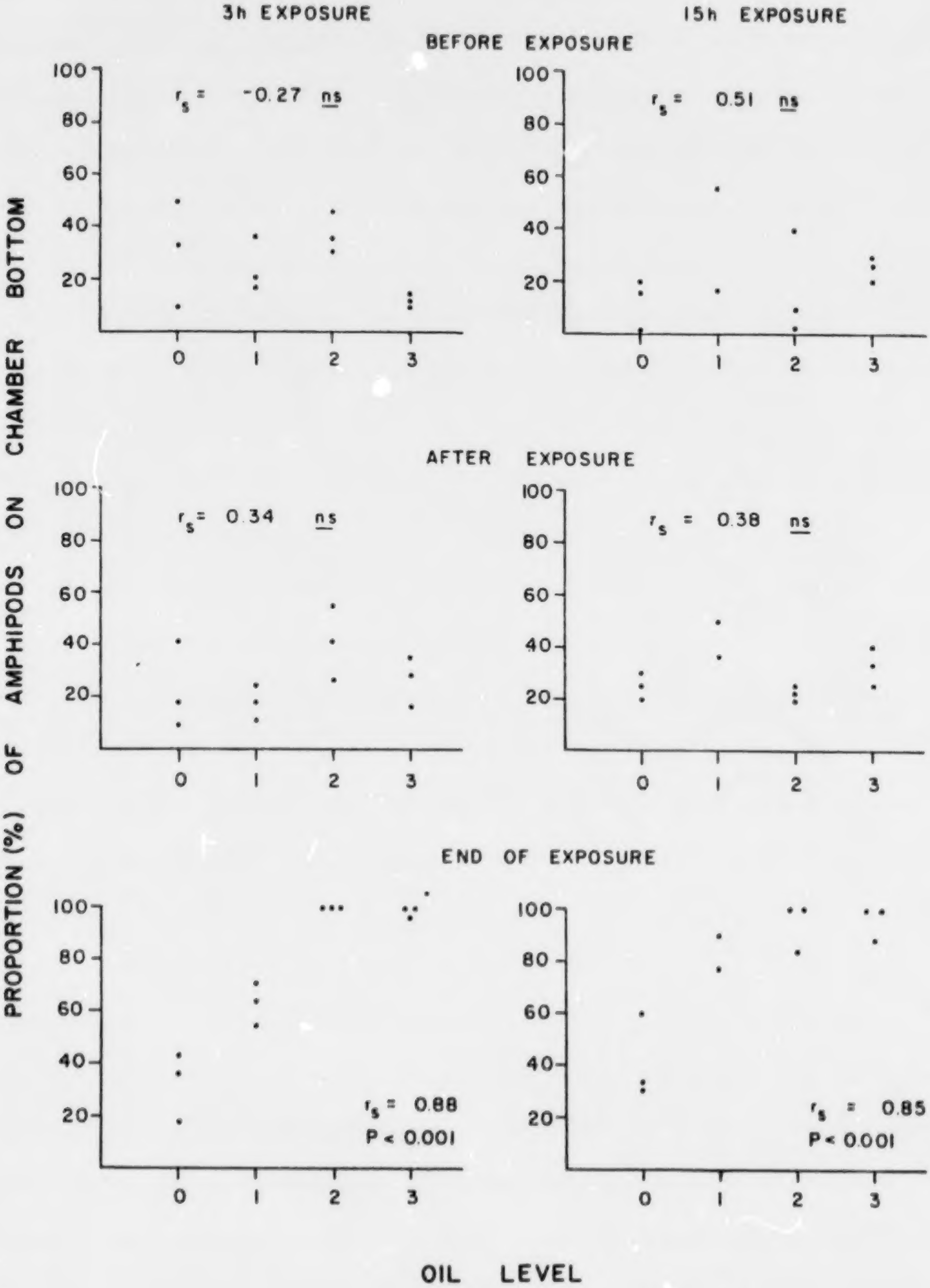

FIGURE 2. Immediate and delayed reaction of amphipods to oil in chambers. Oil levels 0, 1, 2 and 3 represent nominal concentrations of 0, 30, 130 and 400 ppm untreated Lagomedio crude oil.

that at the start of the experiment; a somewhat higher proportion of amphipods were on chamber bottoms at the end of the 15 h exposure. However, at all oil levels in both exposures the percentage of amphipods on the chamber bottoms had increased by the end of the exposure period; at the two higher concentrations (130 and 400 ppm) it was between 80 and 100%. The correlation between amphipod distribution and oil level was highly significant at the end of both experiments ($P<0.001$, Fig. 2).

Some of the amphipods at the chamber bottoms at the end of the exposure period probably were already dead. Those still alive probably were at the chamber bottoms because of impaired locomotory ability or impaired ability to remain on the ice rather than avoidance of the oil on the under-ice surface. Percy and Mullin (1977) reported impairment of locomotory activity in the arctic amphipod Boeckosimus affinis after 24 h exposures to all oil concentrations tested (nominal concentrations of 50-2000 ppm). Our exposures were shorter, but many of the amphipods at all oil levels were apparently unable to swim at the end of the exposure periods.

During our observation periods there was no apparent avoidance of the oil at the under-ice surface. Previous studies have demonstrated an avoidance reaction in Boeckosimus affinis to sediments contaminated with 4 types of oil (Percy 1977a) and to crude oil and oil-tainted food (Percy 1976). The avoidance reaction was diminished, however, if the oil was weathered or if the amphipods were pre-exposed to oil (Percy 1976), or when high concentrations of oil were mixed with sediments (Percy 1977a). Responses varied with type of oil (Percy 1976, 1977a), and Busdosh and Atlas (1977) found a complete lack of an avoidance response to surface slicks of a different type of oil in *B. affinis* and *Gammarus zaddachi*. The importance of this susceptibility to oiling in the case of amphipods living and feeding on the under-ice surface, where oil slicks may accumulate, has already been pointed out (Percy 1974, 1975; Busdosh and Atlas 1977).

Mortality

Data on (1) immediate post-exposure mortality, (2) delayed mortality within four days of exposure, and (3) survival for four days post-exposure, are shown in Tables 2 and 3. Each of these three variables was calculated as a percentage of the total numbers or biomass of amphipods for each chamber (replicate). The three variables are clearly not independent, since the three values for a chamber total 100%; this must be considered in the interpretation of results.

Control mortality immediately following each exposure was low (≤3.3% of numbers, ≤0.2% of biomass; Table 2). Delayed mortality in controls, however, was somewhat higher, particularly following the dispersed oil exposure (31.9% of numbers, 17.4% of biomass). The latter may have been due to the larger numbers of control amphipods maintained in small containers in the laboratory following the dispersed oil exposure (50) as compared with those maintained following exposure to oil alone (20, 27). In any case, delayed control mortality must be attributable to some aspect of the field or laboratory procedures. There was no evidence, however, of any form of contamination of controls by oil during or following exposures.

Table 2. Effects of oil level on immediate and delayed mortality of under-ice amphipods. Data presented are means of percentages within replicate chambers (n = 2 for 15 h exposure, 3 for 3 h exposures).

		Percent of Numbers				Percent of Biomass				ANOVA[1] Significance	
Exposure	Variable	Control	30 ppm	130 ppm	400 ppm	Control	30 ppm	130 ppm	400 ppm	numbers	biomass
Oil-15 h	Immediate mortality	0	25.0	62.9	87.5	0	11.8	41.7	92.2	* P = 0.025	* P = 0.032
	Delayed mortality	14.3	41.7	16.9	8.3	11.9	9.0	20.6	5.3	ns P = 0.344	ns P = 0.646
	Survival	85.7	33.3	20.2	4.2	88.1	79.2	37.6	2.5	* P = 0.022	* P = 0.025
Oil-3 h	Immediate mortality	3.3	3.2	23.3	67.4	0.2	0.8	1.3	36.6	** P = 0.003	ns P = 0.053
	Delayed mortality	7.5	64.5	52.3	27.4	2.1	23.0	52.1	41.7	* P = 0.014	ns P = 0.128
	Survival	89.2	32.3	24.4	5.3	97.7	76.3	46.6	21.7	** P = 0.002	* P = 0.031
Dispersed Oil-3 h	Immediate mortality	0	16.1	15.9	16.1	0	9.5	3.0	8.1	ns P = 0.502	ns P = 0.671
	Delayed mortality	31.9	45.8	27.8	55.2	17.4	39.7	22.3	37.4	ns P = 0.545	ns P = 0.506
	Survival	68.1	38.0	56.2	28.7	82.6	50.8	74.8	54.5	ns P = 0.123	ns P = 0.246

[1] One-factor ANOVA for oil levels, including controls. ns means $P>0.05$, * means $0.05 \geq P>0.01$, and ** means $0.01 \geq P>0.001$.

Table 3. Effects of length of exposure to oil and dispersed oil on immediate and delayed mortality of under-ice amphipods. Each value represents the mean percent mortality in all oiled chambers less mean control mortality; n = 9 except for 15 h exposure where n = 6.

	Oil - 15 h		Oil - 3 h		Dispersed Oil - 3 h		ANOVA Significance	
	% of numbers	% of biomass	% of numbers	% of biomass	% of numbers	% of biomass	numbers	biomass
Immediate mortality	58.5	48.6	28.0	12.7	16.1	6.8	P = 0.023	P = 0.013
Delayed mortality	8.0	-0.3	40.6	36.8	11.0	15.7	P = 0.015	P = 0.011
All mortality	66.5	48.3	68.5	49.5	27.1	22.6	P = 0.001	P = 0.157

Oil Level Effects

Following the 15 hour exposure to oil alone, immediate mortality and survival of Weyprechtia pinguis (based on percent of numbers and of biomass) varied significantly among oil levels (0, 30, 130 and 400 ppm), whereas delayed mortality did not (Table 2). Immediate mortality increased and survival decreased with increased oil level. Following the three hour oil exposure all variables based on numbers differed significantly with oil level; when based on biomass, only survival differed significantly among oil levels, although directions of trends were the same as for numbers. Again, there was a progressive increase in immediate mortality and a progressive decrease in survival with increasing oil level. Delayed mortality generally decreased with increasing oil level, but this trend was simply a result of the lack of independence among variables. Total mortality increased with increasing oil level in both oil-alone experiments.

Foy (1978) also reported increased mortality with increasing oil level in the arctic amphipods Onisimus litoralis and Boeckosimus edwardsii. Oil-seawater dispersions were used in the laboratory, with nominal (added) concentrations of 50-800 ppm and measured concentrations of 20-47 ppm; from the lowest to the highest concentrations, mortality after 96 h exposures increased from 0% to 45% in Onisimus and from 0% to 83% in Boeckosimus. Delayed mortality was not measured, but the condition of the test animals indicated that it could be substantial (Foy 1978). In further studies with Onisimus and other arctic amphipods, additional mortality was indeed observed in 24 h post-exposure periods (Foy 1979).

Following the three hour exposure of Weyprechtia pinguis to dispersed oil, differences among oil levels (including controls) were not significant for any variable ($P \geq 0.123$ in each case; Table 2). This was only partly attributable to relatively high mortality in controls. Survival in Weyprechtia at high oil levels was consistently higher when the amphipods had been exposed to dispersed oil then when exposed to untreated oil. The trends apparent after exposure to untreated oil (progressive increases or decreases with increasing oil level) were not apparent in any variable after exposure to dispersed oil. The results of Foy (1978) for short exposures to Corexit-dispersed oil are similar: very little mortality was observed after 24 h laboratory exposures at concentrations up to 213 ppm (nominal concentration of 400 ppm) in the amphipods Onisimus litoralis and Boeckosimus edwardsii. At a higher concentration (800 ppm nominal, 355 ppm measured), however, 24 h mortality was >50% in both species.

Exposure Effects

The type of exposure had a significant effect on the amount and timing of mortality (Table 3). One demonstration of this is the fact that there was a significant 'oil level x exposure type' interaction term in 2-way ANOVAs of immediate mortality ($P = 0.005$ for biomass, $P = 0.011$ for numbers). Immediate mortality was highest following the 15 hour exposure to oil alone (58.5% of numbers, 48.6% of biomass) and delayed mortality was highest following the three hour exposure to oil alone (40.6% of numbers, 36.8% of biomass). Total mortality was similar following 15 or 3 h exposures to undispersed oil (66.5, 68.6% of numbers; 48.3, 49.5% of biomass), but much greater than total mortality after 3 h exposure to dispersed oil at the same three oil levels. Total mortality after exposure to dispersed oil was less than half of that following exposure to oil alone (Table 3).

Size effects

Differences between mean size of *Weyprechtia pinguis* in the sample and mean size of those dying within four post-exposure days were small (dead amphipods were smaller by an overall average of 1.0 mm); mean size of dead *Weyprechtia* was greater than mean size of the whole sample in only two of 28 cases, and smaller in 21 of 28 cases. There were no significant differences in this variable (the difference in mean size between all amphipods and dead amphipods), however, either among exposures (P = 0.89) or among oil levels, including controls (P = 0.39). This suggests that any size selective mortality that occurred was due to handling, either *in situ* or in the laboratory.

Degree of Oiling vs. Mortality

The relationship between mortality and degree of oiling (i.e. evidence of oil on preserved amphipods) was examined using a contingency table. Only oil levels 1, 2 and 3 were considered (89.4% of these amphipods were oiled to some extent); the inclusion of control data could produce a significant relationship merely because no control amphipods were oiled. As dispersed oil did not adhere to amphipods, only data from 'oil alone' exposures were used.

The resultant contingency table is shown in Table 4. At higher levels of oiling (right half of table), immediate mortality was not related to extent of oiling; none of these amphipods survived beyond the end of the exposure. In order to evaluate the effects of low to moderate oiling on survival, statistical tests were carried out only on the left half of the contingency table (low to intermediate degree of oiling). The χ^2 statistic for this table was highly significant (df = 4; P = 0.0001). Immediate mortality increased and survival for any length of time decreased with increased oiling when only the low to moderate levels of oiling were considered.

The results of the present field study on the effects of untreated oil on under-ice amphipods corroborate those of Percy (1974) and Busdosh and Atlas (1977), who concluded from laboratory studies that amphipods that come into direct contact with oil have little chance of survival. Amphipods either have no apparent avoidance response to oil (present study; also Busdosh and Atlas 1977), or an observed avoidance response is diminished in the presence of high concentrations of oil (Percy 1976, 1977a). This further indicates the susceptibility of these animals to oil spills. This study extends the results of laboratory experiments to actual conditions on the under-ice surface, and supports the concern of previous investigators (e.g., Percy 1974, 1975; Busdosh and Atlas 1977; Foy 1978) that under-ice communities may be particularily susceptible to oil spills.

The use of chemical dispersants increases the dispersion of oil in water, generally leading to higher mortalities in test organisms for a given amount of oil added (e.g., Foy 1978, 1979). These previous results are based

Table 4. Degree of oiling vs. immediate mortality, delayed mortality and survival of *Weyprechtia pinguis*. 'Degree of oiling' is the sum of oil codes (light, medium, heavy = 1, 2, 3) on 8 body parts (see 'Methods') of each amphipod exposed to oil alone (controls not included).

	Degree of Oiling						
	0-3	4-7	8-11	12-15	16-19	20-24	Total
Immediate mortality	6	22	30	17	17	11	103
Delayed mortality	56	25	3	0	0	0	84
Survival	39	3	0	0	0	0	42
Total	101	50	33	17	17	11	229

on relatively long (24-96 h) exposures to mechanically dispersed oil-water or dispersant-oil-water mixtures, however, and thus do not simulate a realistic spill situation. In the present study, amphipods exposed in situ to various concentrations of Corexit-oil-water mixtures for a relatively short (3 h) period of time were less affected than were those exposed to oil masses together with low concentrations of dissolved oil components for relatively short (3 h) or long (15 h) periods of time. Sublethal effects on metabolism, activity or nutritional state (Percy 1977b) were not studied, but our results on immediate mortality and survival for a 4-day post exposure period indicate that the chemical dispersion of oil may be less harmful to amphipods in the under-ice community than the accumulation of untreated oil on the ice undersurface. Dispersed oil may contact much larger areas of the under-ice surface, however, resulting in greater total mortality. Further studies are required on the relative toxicities of dispersed and untreated oil at realistic concentrations, and, in particular, on the behaviour of oil and dispersed oil under the ice.

Under-ice Amphipod Distribution and Population Structures

Site Description

Under-ice amphipods were sampled in Bays 9, 10 and 11 at Cape Hatt (Fig. 1) through dive holes over a water depth of 3-4 m. In each bay, holes were located just offshore (within 10 m) of the furthest tide crack from shore; the area sampled was seaward of the dive hole and consisted of smooth, relatively flat ice. Ice depths at the dive holes were 122, 152 and 163 cm in Bays 9, 10 and 11, respectively, and snow depths on 31 May were 14.3 ± SD

4.2 cm (n = 39), 14.3 ± 4.9 cm (n = 27) and 8.9 ± 5.5 cm (n = 39). In each bay a relatively light-coloured algal layer was present on the ice undersurface, and its distribution was patchy on a scale of metres or tens of metres.

Intertidal and shallow sublittoral substrates consisted of fine to coarse sand mixed with pebble and cobble; finer sediments became increasingly predominant with increasing depth. The shoreline was icebound to a depth of 2 or 3 m during May, and very little drifting or grounded ice was present during August and September.

The under-ice macrofauna in the three study bays included arctic cod (Boreogadus saida) and mysids (Mysis spp.), but otherwise consisted entirely of gammarid amphipods. Cod were observed only in the large tide cracks just inshore of the entry holes in each of the bays. The edges of these cracks were rounded and about 1/2 m apart at the bottom, and only a few cm wide at a distance of about 1/2 m from the bottom of the ice; most cod moved up into the cracks when disturbed. Mysids were present throughout the water column in each bay, and were generally concentrated in the first metre of water just below the ice. Densities of mysids were extremely variable both within and among bays; estimates based on amphipod dip net samples were as high as 225 individuals or 6 g/m^2. These are underestimates, probably by at least an order of magnitude, because our nets sampled only a part of the water column and because mysids actively avoided the nets. The following results concern only the amphipods collected in this study, and only those amphipods occurring on the under-ice surface are treated in detail.

Species Composition

Ten species of gammarid amphipods were collected on the under-ice surface at Cape Hatt (Table 5). Two species, *Ischyrocerus* sp. and *Weyprechtia pinguis*, together accounted for 61.9% of total numbers, and three species, *Weyprechtia pinguis*, *Gammarus setosus* and *Onisimus litoralis*, together accounted for 86.1% of total biomass. Eight of the 10 species have previously been collected in spring on the under-ice surface at various localities in the central and eastern Arctic (Green and Steele 1975; Buchanan et al. 1977; Thomson et al. 1978; Cross 1980, 1982); the exceptions were *Weyprechtia pinguis* and *Pontogenieia inermis*. Those two species have previously been collected on the under-ice surface only in late February in the western Arctic (Griffiths and Dillinger 1981).

By comparison, species diversity was considerably lower in the intertidal habitat (samples from August and September 1981) and considerably higher in the sublittoral habitat (samples from May, August and September 1981). Four amphipod species were found in the intertidal habitat; *Gammarus setosus* was dominant both in terms of numbers (93.2% of total) and biomass (76.8% of total). In the shallow (3-4 m) sublittoral habitat, 31 identified species and at least 7 distinct but unidentified species were collected. *Orchomene minuta*, *Guernea* sp. and *Protomedia fasciata* together accounted for 50.7% of total numbers collected in May, August and September, and *Anonyx nugax*, *Anonyx sarsi*, *Orchomene minuta* and *Paroediceros lynceus* together accounted for 62.9% of total biomass.

All four species found in the intertidal habitat were also found on the under-ice surface. Three and seven of the 10 amphipod species found under

Table 5. Species composition (% of total numbers and biomass) of amphipods in under-ice, intertidal and benthic habitats in three bays at Cape Hatt during May, August and September 1981.

Species	Intertidal (Aug, Sept)		Under-ice (May)		Sublittoral (May)		Sublittoral (Aug, Sept)	
	% of numbers	% of biomass	% of numbers	% of biomass	% of numbers	% of biomass	% of numbers	% of biomass
Onisimus litoralis	3.8	14.0	8.0	28.5			1.8	10.6
Gammarus wilkitzkii	0.7	4.6	0.1	1.1			<0.1	0.1
Gammarus setosus	93.2	76.8	8.8	26.3				
Gammaracanthus loricatus	0.2	4.1	0.5	1.5				
Ischyrocerus sp.			41.5	7.6	<0.1	<0.1	0.2	<0.1
Weyprechtia pinguis			20.4	31.3	<0.1	0.5	6.2	11.9
Onisimus glacialis			1.8	1.9			0.2	0.5
Pontogeneia inermis			0.2	0.2	0.3	0.6	0.2	<0.1
Onisimus nanseni			<0.1	0.1			<0.1	0.5
Apherusa glacialis			1.0	0.4				
Orchomene minuta					25.0	24.8	15.4	7.8
Guernea sp.					27.3	3.5	10.7	0.7
Protomedeia fasciata					13.6	2.5	3.2	0.7
Paroediceros lynceus					4.7	8.5	9.9	12.7
Monoculopsis longicornis							14.0	0.9
Monoculodes borealis					0.7	3.3	12.2	4.7
Boeckosimus plautus					2.5	2.6	4.9	4.4
Corophium clarenscense					3.9	0.6	0.3	<0.1
Stenothoidae spp.					0.9	0.2	4.2	0.3
Monoculodes latimanus					3.3	2.1	0.7	0.3
Calliopiidae spp.					2.4	1.1	1.7	0.2
Oedicerotidae spp. (2 species)					0.1	<0.1	2.9	0.2
Anonyx nugax					1.2	34.6	0.9	11.1
Portopereia femorata					1.0	2.2	1.2	0.6
Oediceros borealis					1.0	0.5		
Anonyx sarsi					<0.1	0.3	1.2	28.3
Westwoodilla megalops					0.4	<0.1	<0.1	<0.1
Monoculodes longirostris					0.3	1.7		
Atylus carinatus							0.2	1.1
Bathymedon obtusifrons					0.2	<0.1		
Melita dentata					0.1	0.1		
Monoculodes schneideri							0.3	0.2
Boeckosimus edwardsii							<0.1	<0.1
Lysianassidae sp.					<0.1	<0.1		
Monoculodes packardi							<0.1	<0.1
Phoxocephalus holbolli					<0.1	<0.1		
Westwoodilla brevicalcar							<0.1	<0.1
Total %[1]	97.9%	99.5%	82.3%	98.9%	88.9%	89.7%	92.5%	97.8%
Total number or biomass (g)	969	16.312	4127	49.122	1782	7.549	1268	13.249
Total no./m^2 or g/m^2	194	3.262	13	0.154	1188	5.033	423	4.416

[1] Does not include unidentified, juvenile or damaged amphipods.

the ice also occurred in samples from the shallow sublittoral habitat in spring and summer, respectively (Table 5). *Gammarus setosus* and *Gammaracanthus loricatus* were not collected on the bottom (but were present in the intertidal habitat); these species have previously been reported to occur in the nearshore benthos (e.g., MacGinitie 1955; Steele and Steele 1970) and were found in low numbers in samples collected by Cross and Thomson (1981, 1982) at 3 and 7 m depths in the same bays at Cape Hatt (LGL Ltd., unpubl. data). *Apherusa glacialis* formed a small part of the under-ice community, but was found in neither intertidal nor sublittoral habitats, either in this study or in that of Cross and Thomson (1982). This species is pelagic (Dunbar 1954) and often associates with pan ice in late summer (Stephensen 1942; MacGinitie 1955; Divoky 1978; Thomson et al. 1978). Species occurring on the under-ice surface in May were of very little importance in the sublittoral habitat in May (0.3% of numbers, 1.1% of biomass), and considerably higher importance in August and September (8.6% of numbers, 23.6% of biomass; Table 5).

Percent composition of total numbers and biomass of under-ice amphipods in each bay during mid-May and late May are shown in Figure 3. Differences among bays were more marked than differences between mid- and late May. In terms of numbers, percent composition changed very little from mid- to late May. In terms of biomass, the most obvious temporal difference is the reduced relative importance of *Weyprechtia pinguis* in late May.

In terms of numbers, *Weyprechtia pinguis* and *Ischyrocerus* sp. were the most important species on the under-ice surface. At both times in May, *Weyprechtia* was dominant in Bay 9 and *Ischyrocerus* was dominant in Bays 10

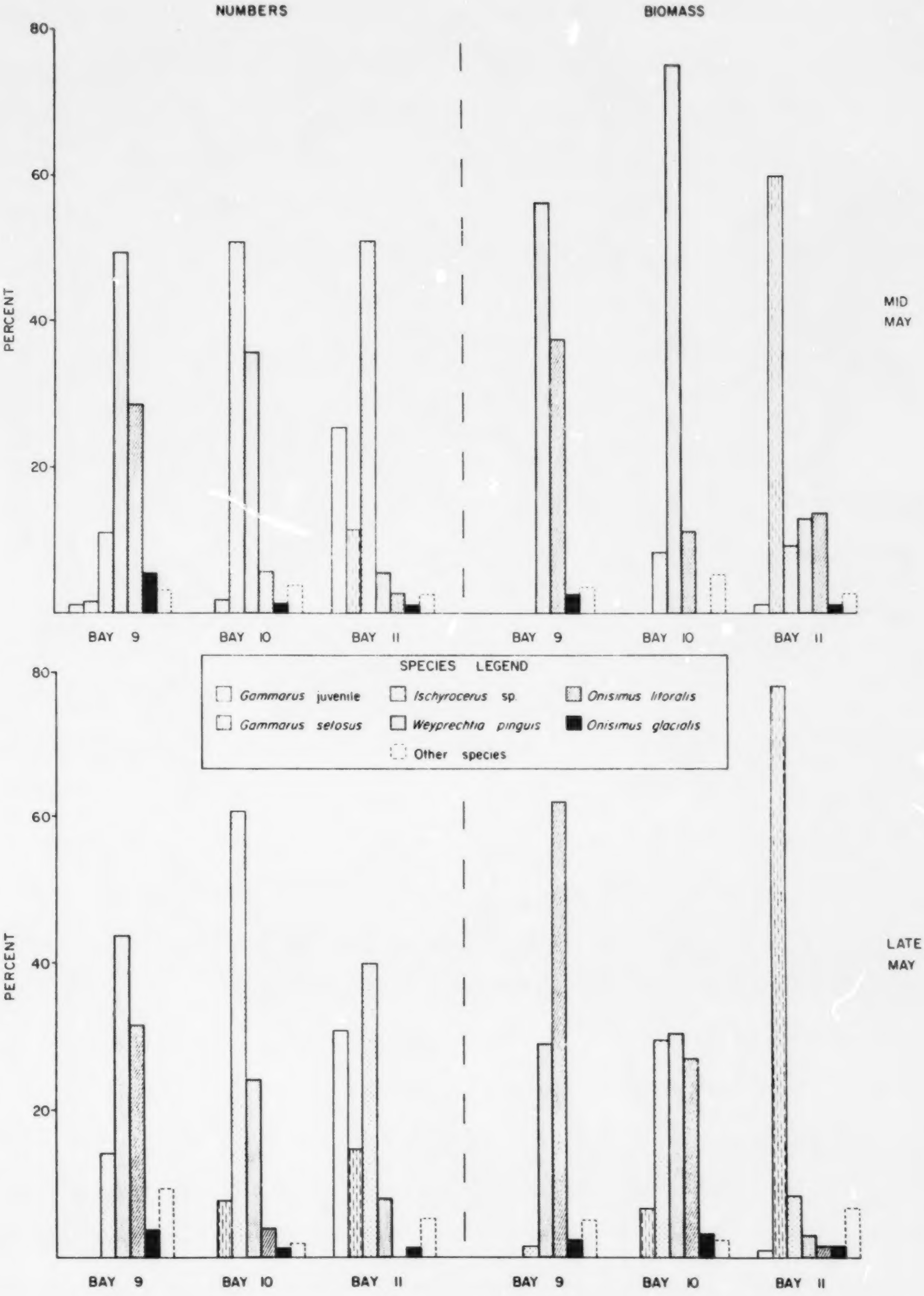

FIGURE 3. Percent composition of numbers and biomass of under-ice amphipods in systematic net samples collected during 17-19 May and 31 May 1981 in three bays at Cape Hatt, Baffin Island.

and 11; both species were among the top 3 contributors to total numbers in each bay at each time. *Onisimus litoralis* and *Onisimus glacialis* comprised higher percentages of numbers in Bay 9 than in Bays 10 or 11. *Gammarus setosus* and *Gammarus* juveniles together accounted for 37.0% and 45.3% of total numbers in Bay 11 in mid- and late May, respectively, and contributed much less (<9%) to total numbers in Bays 9 and 10.

In terms of biomass, *Ischyrocerus* was much less important (Fig. 3); at both times in May, *Weyprechtia* was dominant in Bay 10 and *Gammarus setosus* was first-ranked in Bay 11. *Onisimus litoralis* was more important in Bay 9 than in Bays 10 or 11, and was dominant (62.1% of biomass) in Bay 9 on 31 May.

Abundance and Biomass

Abundance (nos./m^2) and biomass (g/m^2) of all amphipods in under-ice, intertidal and sublittoral habitats are shown for each of the 3 bays and 4 sampling periods in Figure 4. In general, abundance and biomass were highest in the sublittoral habitat, intermediate in the intertidal habitat, and lowest on the under-ice surface. However, intertidal habitats sometimes contained more amphipods than sublittoral ones. From August to September, sublittoral abundance and biomass remained similar or increased in most cases, whereas intertidal amphipods usually decreased (Fig. 4).

Considerably fewer amphipods occurred on the under-ice surface in mid-May (<15/m^2) than in the sublittoral habitat at the same time (>200/m^2); differences in biomasses were less marked, but still very considerable.

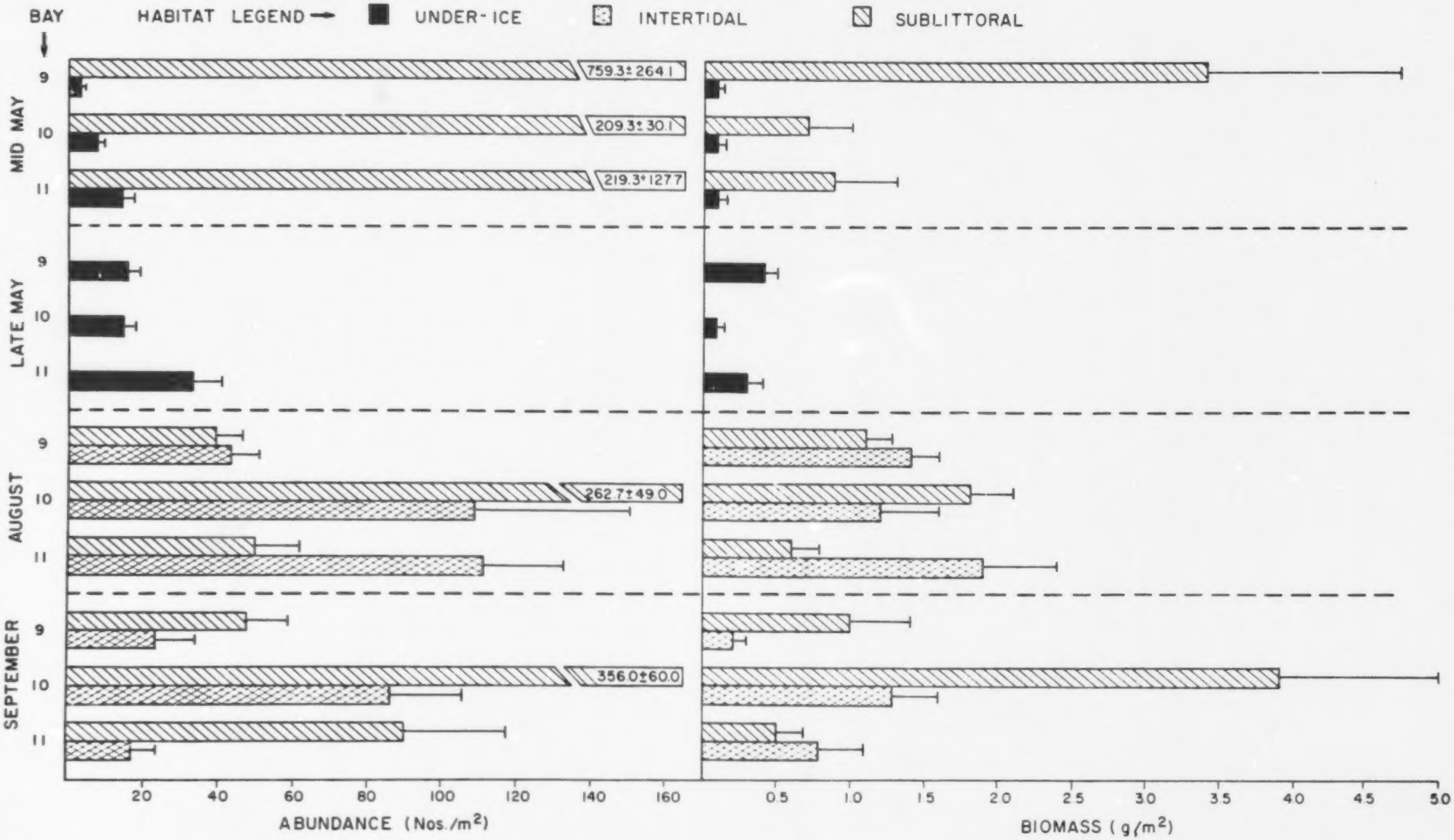

FIGURE 4. Abundance and biomass of all amphipods in under-ice, intertidal and sublittoral habitats in three bays and at four times at Cape Hatt, Baffin Island. Data are given as mean (boxes) ± S.E. (lines).

However, the species that dominated the under-ice habitat were more numerous there than on the bottom. Four dominant species, *Ischyrocerus* sp., *Weyprechtia pinguis*, *Onisimus litoralis* and *Gammarus setosus*, were present on the under-ice surface in relatively low abundances in mid-May (0.1 to 7.1 indiv./m^2). Of these, only *Ischyrocerus* and *Weyprechtia* were collected in the sublittoral habitat at that time, and then only in one bay (Table 6); for *Ischyrocerus*, this was the only occurrence in the sublittoral samples. In this bay, numbers of *Weyprechtia* in the sublittoral habitat increased from May to August, and decreased in September; in Bays 10 and 11, *Weyprechtia* was absent in sublittoral samples in May, present in August, and again absent in September (Table 6). Similarly, *Onisimus* was present in the sublittoral habitat only in August. *Gammarus* was not present in samples from the sublittoral habitat; it was found on the ice in all three bays, but was common only in Bay 11. These data indicate a very strong preference for the under-ice habitat in May for the four dominant species on the under-ice surface. This preference apparently varies among species and with locality: Percy (1975) reported that *Boeckosimus affinis* congregates near the ice in winter, but is still present in the sediment, and leaves the ice in late May to return to the bottom. Griffiths and Dillinger (1981) reported that *Weyprechtia pinguis* was collected both on the bottom and on the under-ice surface in Prudhoe Bay in late February.

A comparison of under-ice and intertidal habitats also shows species and locality differences. *Gammarus setosus* was present either on the under-ice surface (May) or in the intertidal habitat (August, September) in all 3 bays; in each case abundances were highest in August and lowest in May. Densities were considerably higher in the intertidal area (12.8 to 102.4 indiv./m^2

depending on bay and month) than on the under-ice surface (0.1 to 4.8 indiv./m^2). *Onisimus litoralis* was also present in each bay on the under-ice surface in May, and was found in the intertidal area in August and September, but only in Bays 10 and 11. Densities were similar in both habitats in Bay 10, and higher in the intertidal habitat in Bay 11. *Weyprechtia pinguis* and *Ischyrocerus* sp. were absent from the intertidal areas of all three bays (Table 6).

Analyses of variance were used to compare abundances and biomasses of all amphipods and of four dominant species in different bays and at different times (Table 7). In most two-way ANOVA's, interactions between the bay and time factors were significant ($P<0.05$; Table 7). This indicates that differences in abundance or biomass from one time to another (mid-May to late May, or August to September) were not consistent among bays; conversely, differences among bays were not consistent from one time to another. These interactions necessitated the use of separate one-factor ANOVAs (bay effects) for each time period.

In general, there were pronounced among-bay differences in abundances and biomasses of total amphipods and dominant species of amphipods. Among-bay differences were somewhat more pronounced in late May than in mid-May: in mid-May there were no differences among bays in the biomass of total amphipods, or in the density or biomass of *Onisimus litoralis*, whereas in late May densities of all taxa and biomasses of all taxa but *Ischyrocerus* sp. differed significantly among bays. In the intertidal habitat, the numbers and biomass of *Onisimus litoralis* differed significantly among bays only in August, whereas numbers and biomass of *Gammarus setosus* differed among bays only in September (Table 7).

Table 6. Densities (nos./m^2) of dominant under-ice amphipod species in under-ice, intertidal and shallow sublittoral habitats during May, August and September 1981 in three bays at Cape Hatt, Baffin Island. Data are expressed as mean ± SD and based on 10-16 replicates.

Species	Habitat	Bay 9				Bay 10				Bay 11			
		Mid May	Late May	Aug	Sept	Mid May	Late May	Aug	Sept	Mid May	Late May	Aug	Sept
Weyprechtia pinguis	Under-ice/Intertidal[1]	1.5 ± 1.0	6.7 ± 5.2	0	0	2.6 ± 3.5	3.4 ± 3.6	0	0	0.8 ± 1.5	2.6 ± 3.1	0	0
	Shallow Sublittoral[2]	0.7 ± 2.1	-	9.3 ± 11.4	1.3 ± 2.8	0	-	41.3 ± 26.1	0	0	-	0.7 ± 2.1	0
Onisimus litoralis	Under-ice/Intertidal	0.9 ± 1.2	4.9 ± 4.7	0	0	0.4 ± 0.8	0.6 ± 0.7	0.8 ± 1.7	0.4 ± 1.3	0.3 ± 1.0	0.1 ± 0.3	12.8 ± 7.3	0.8 ± 1.7
	Shallow Sublittoral	0	-	4.7 ± 7.1	0	0	-	2.0 ± 3.2	0	0	-	8.7 ± 9.5	0
Ischyrocerus sp.	Under-ice/Intertidal	0.3 ± 0.3	1.2 ± 1.5	0	0	3.7 ± 3.2	8.6 ± 10.2	0	0	7.1 ± 7.4	13.1 ± 9.9	0	0
	Shallow Sublitoral	0.7 ± 2.1	-	0	0	0	-	0	0	0	-	0	0
Gammarus setosus	Under-ice/Intertidal	0.1 ± 0.1	0.1 ± 0.1	42.0 ± 21.6	23.6 ± 34.1	0.1 ± 0.2	1.1 ± 1.9	102.4 ± 129.4	85.2 ± 61.4	1.6 ± 1.7	4.8 ± 3.1	95.2 ± 58.9	12.8 ± 20.6
	Shallow Sublittoral	0	-	0	0	0	-	0	0	0	-	0	0
Total amphipods	Under-ice/Intertidal	2.9 ± 2.0	15.3 ± 11.8	43.2 ± 22.7	23.6 ± 34.1	7.3 ± 5.7	14.1 ± 12.2	108.0 ± 135.0	86.4 ± 60.6	13.9 ± 11.9	32.5 ± 24.5	111.6 ± 67.5	16.8 ± 21.7
	Shallow Sublittoral	759.3 ± 835.2	-	39.3 ± 23.0	47.3 ± 35.1	209.3 ± 95.0	-	262.7 ± 155.0	356.0 ± 190.0	219.3 ± 404.0	-	50.0 ± 36.8	90.0 ± 85.9

- no samples collected.

[1] Under-ice in May and intertidal in August and September.

[2] Depth 3-5 m.

Table 7. Comparison of biomasses and densities of amphipods in under-ice and intertidal habitats in three bays at two times at Cape Hatt, Baffin Island. F - values are shown with associated significance levels (ns = $P>0.05$; * $P\leq0.05$; ** $P\leq0.01$; *** $P\leq0.001$). In the 2-way ANOVAs, significance levels are not given for the main effects when the interaction term was significant.

			Source of variation and df[1]				
			Two-factor ANOVA			One-factor ANOVA (bays)	
Habitat	Variable	Taxon	Bay 2,74(54)	Time 1,74(54)	Bay x Time 2,74(54)	Bay[2] 2,45(27)	Bay[3] 2,45(27)
Under-ice Habitat	Biomass	Total amphipods	2.16	14.92	6.73 **	0.42 ns	5.91 **
		Gammarus setosus	32.82	10.01	9.06 ***	15.69 ***	18.99 ***
		Onisimus litoralis	12.24	12.09	12.11 ***	1.10 ns	11.88 ***
		Weyprechtia pinguis	6.77	0.08	5.25 **	4.07 *	13.01 ***
		Ischyrocerus sp.	6.76 **	8.12 **	1.22 ns	6.66 **	2.78 ns
	Abundance	Total amphipods	9.38 ***	23.24 ***	1.96 ns	8.54 ***	3.35 *
		Gammarus setosus	63.35	25.49	6.89 **	35.08 ***	31.25 ***
		Onisimus litoralis	21.23	13.24	12.37 ***	2.14 ns	30.98 ***
		Weyprechtia pinguis	5.70 **	14.48 ***	2.17 ns	4.05 *	3.37 *
		Ischyrocerus sp.	23.19 ***	6.91 *	0.24 ns	14.08 ***	9.14 ***
Intertidal Habitat	Biomass	*Gammarus setosus*	0.20	3.22	6.34 **	2.07 ns	4.81 *
		Onisimus litoralis	29.98	24.40	21.95 ***	31.79 ***	1.04 ns
	Number	*Gammarus setosus*	1.34	6.90	9.92 ***	1.90 ns	11.12 ***
		Onisimus litoralis	29.68	23.43	18.69 ***	36.38 ***	1.08 ns

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[1] Denominator df are shown for under-ice habitat, followed by intertidal habitat (in parentheses).

[2] Bay effects for mid-May (under-ice) or August (intertidal).

[3] Bay effects for late May (under-ice) or September (intertidal).

Lagomedio crude oil was spilled in Bay 11 between the August and September sampling periods, and a relatively even coating of oil was deposited on the beach (intertidal area) of Bay 11 by the falling tide. Observations following the spill (Cross and Thomson 1982), inspection of the data (Table 6) and significance of the bay x time interaction terms in Table 7 all indicate that oil affected the amphipods that occupied the intertidal habitat. The numbers of intertidal Gammarus setosus decreased somewhat in Bays 9 and 10 from August to September, but the corresponding decrease in Bay 11 was much more marked (September densities were 56.2, 83.2 and 13.4% of August values in Bays 9, 10 and 11, respectively). Onisimus litoralis was not found in the intertidal habitat in Bay 9, and was very sparsely distributed in Bay 10, but, like Gammarus, numbers decreased drastically in the intertidal area of Bay 11.

The absence or low abundance of Gammarus setosus in the sublittoral habitat, and its abundance in the intertidal habitat, indicate that the latter is the source of recruitment for this species to the under-ice habitat. Thus, Gammarus may be less abundant on the ice in Bay 11 in 1982 than in 1981. Sampling of all 3 habitats is continuing in 1982, and any effects of the summer spills on the under-ice communities in the following spring, as well as any recovery in the intertidal zone, should be particularly easy to detect for this species in Bay 11.

Population Structures

Length-frequency histograms for Weyprechtia pinguis and Onisimus litoralis collected on the under-ice surface in May, and in intertidal

(*Onisimus*) or sublittoral (*Weyprechtia*) habitats in August, are shown in Figure 5. Both species are of a similar size, and two cohorts (year classes) are apparent for each. Too few of the first year class of *Onisimus* were collected to warrant analysis or discussion.

The second year class of *Onisimus* increased significantly in size from 12.2 to 15.2 mm over the mid-May to late May to August study period ($F = 34.57$, $df = 2,30$; $P<0.0001$), but only the increase from May to August was significant (Duncan's Multiple Range Test, $P<0.05$). Two-factor ANOVA (bays, times) for *Onisimus* from the under-ice surface only, showed no significant differences in size either between the two May sampling periods ($F = 3.54$, $df = 1,18$; $P = 0.076$) or among the bays ($F = 1.06$, $df = 2,18$; $P = 0.368$).

The first year class of *Weyprechtia pinguis* was considerably more abundant in late May than in mid-May, and the reverse was true for the second year class (Fig. 5; areas sampled were 576 m^2 and 384 m^2 in mid- and late May, respectively). This indicates that release of juveniles was occurring between the two dates. Juveniles increased significantly in length over the mid-May to August study period, from 3.6 to 7.2 mm ($F = 155.27$, $df = 2,36$; $P\leq 0.0001$), and significant growth occurred in both the mid-May to late May and the late May to August periods (Duncan's Multiple Range Test, $P<0.05$). Similarily, the second year class of *Weyprechtia* increased significantly in length over the study period ($F = 23.01$, $df = 2,33$; $P\leq 0.0001$). Results of two-factor ANOVA and multiple comparisons showed that the first year class was significantly smaller in Bay 11 than in Bays 9 and 10 ($F = 8.87$, $df = 2,26$; $P = 0.0012$), and that the second year class decreased progressively in size from Bay 9 to Bay 10 to Bay 11 ($F = 5.44$, $df = 2,28$; $P = 0.0101$).

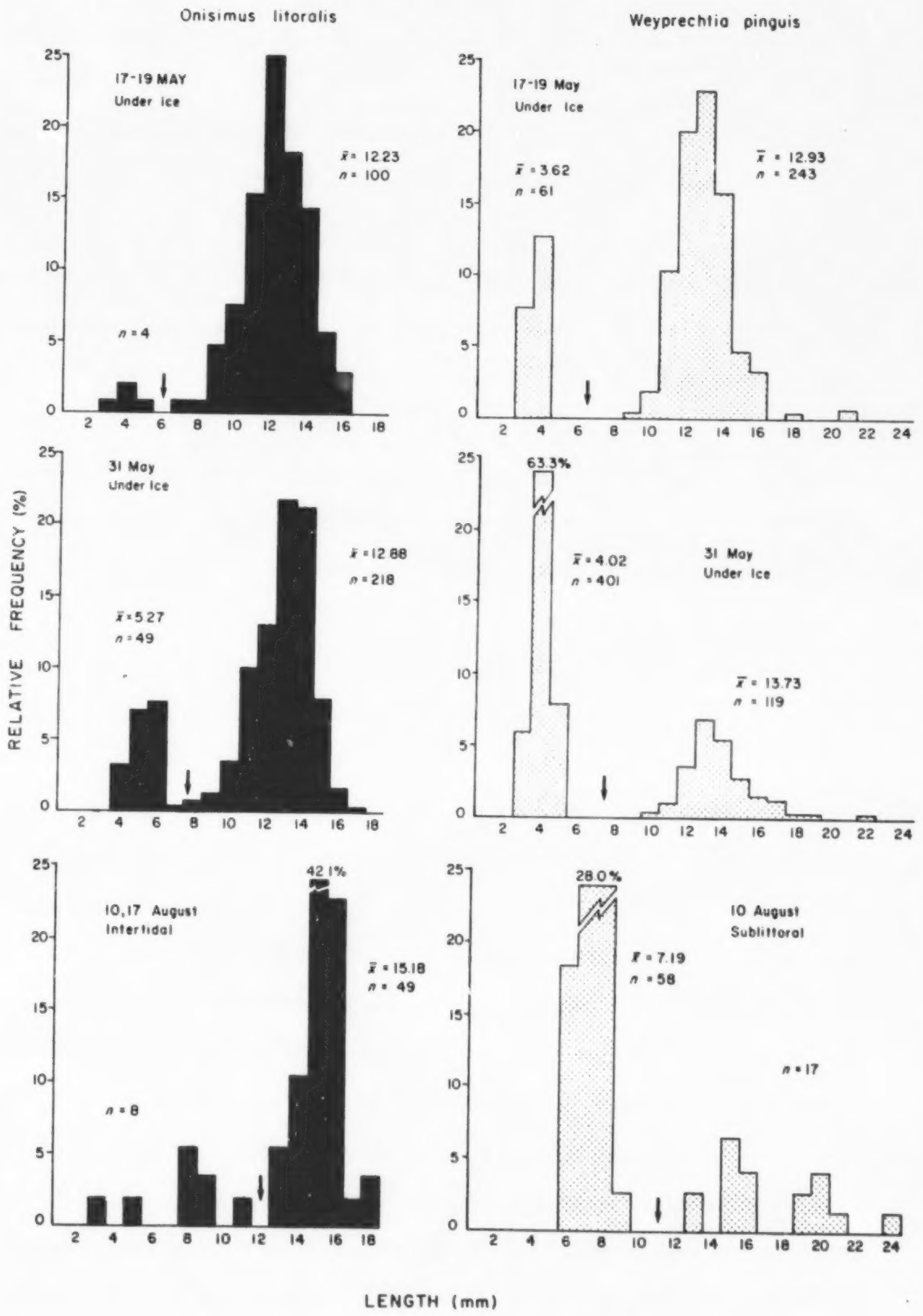

FIGURE 5. Length-frequency histograms for Onisimus litoralis and Weyprechtia pinguis in under-ice (May) and intertidal or sublittoral (August) habitats at Cape Hatt, Baffin Island, in 1981. Arrows indicate divisions between cohorts for mean size calculations.

Length-frequency histograms for *Gammarus setosus* (+ *Gammarus* juveniles) collected on the under-ice surface in mid- and late May, and in the intertidal habitat in August and September, are shown in Figure 6. At least three cohorts are present; the largest probably consists of two or more year classes, but numbers are too low to allow distinction or further discussion. The smallest cohort had apparently been released prior to our first sampling period. These juveniles grew significantly, from 3.0 to 5.7 mm on average, between mid-May and mid-September ($F = 204.7$, $df = 3,44$; $P<0.0001$). Growth was not apparent between the two sampling dates in May, likely because amphipods were measured only to the nearest mm. Mean sizes were significantly different, however, in each of May, August and September (Duncan's Multiple Range test; $P<0.05$). The second year class increased in length from 7.1 to 12.8 mm during the study period (Fig. 6). Again, this increase was significant ($F = 144.5$, $df = 3,36$; $P<0.0001$), but for this year class, increase in length was significant between each of the sampling dates.

Two-factor (bays, months) ANOVA for mean size data on *Gammarus setosus* collected in the intertidal habitat showed non-significant interaction effects between bay and month factors in either cohort ($F = 1.93$, $df = 2,33$; $P = 0.1617$ for cohort 1 and $F = 0.14$, $df = 2,13$; $P = 0.8673$ for cohort 2). There were also no differences among bays in the mean sizes of *Gammarus* in either cohort ($F = 2.26$, $df = 2,33$; $P = 0.1202$ for cohort 1 and $F = 1.91$, $df = 2,33$; $P = 0.1877$ for cohort 2). These results, together with inspection of Figure 6, indicate that there was no size-selective mortality in *Gammarus* resulting from the spill in late August.

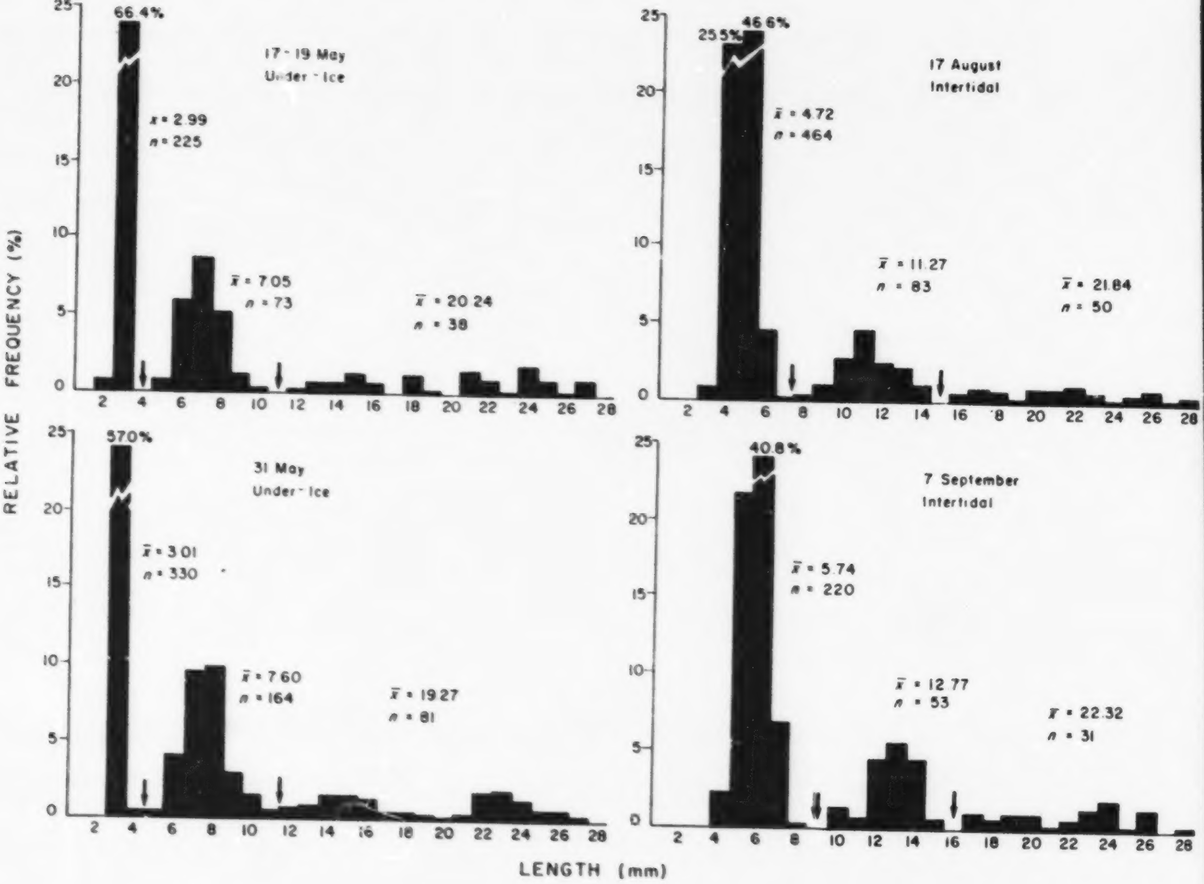

FIGURE 6. Length-frequency histograms for *Gammarus setosus* in under-ice (May) and intertidal (August, September) habitats at Cape Hatt, Baffin Island, in 1981.

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